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the guise of hypothyroidism / **6**

a "diagnostic map" that's hard to read

The symptoms of classical myxedema actually provide only a few sign posts in the vast territory of hypothyroidism. To find the path of correct treatment, "a high degree of suspicion for thyroid disorder remains the most important factor in diagnosis of any case."[1]

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METABOLISM OF 4-C^{14}-CORTICOSTERONE IN MAN*†

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CORTISOL (17-hydroxycorticosterone) is presently accepted as being the main corticosteroid secreted by the adrenal gland of man. It has been recently isolated from human adrenal vein blood by Romanoff *et al.* (1) and evidence has been presented that it is the main free corticosteroid in the peripheral plasma of adult human subjects (2, 3).

Corticosterone, however, has also been isolated from human adrenal vein blood (1). It is probably present in peripheral plasma as well, but the value of the ratio of cortisol to corticosterone has been the subject of controversy; Morris and Williams (4) and Sweat *et al.* (5) reported ratios of approximately 1, whereas Bush and Sandberg (2) and Pincus and Roman-

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off (6) reported much higher values. Whatever the relative concentration of these two steroids really is, it was thought important to study the metabolism of 4-C^{14}-corticosterone in man and, more especially, to compare it with that of 4-C^{14}-cortisol.

METHODS

4-C^{14}-corticosterone was provided by the Endocrine Study Section, Division of Research Grants, National Institutes of Health, Bethesda, Maryland. Its specific activity was reported to be 1.467 mc./m.mole. Data supplied by H. Levy and S. Kushinsky of the Worcester Foundation showed that the sample was 95–96 per cent pure. In our hands, using a similar technique but with the chromatographic method of Bush (7) and the system of solvents benzene/methanol:water (55:45 by volume), approximately 92 per cent of the radioactivity was found in the area of the paper strip corresponding to corticosterone.

The radioactive steroid was dissolved in redistilled ethanol so that 2 ml. of the solution contained approximately 1.5 microcuries, and was kept in the refrigerator. For human administration, a 2-ml. aliquot of this solution was carefully pipetted out and diluted with 15 ml. of sterile isotonic saline solution. The method of intravenous injection and the mode of calculation of the dose actually administered were similar to those described for 4-C^{14}-cortisol (8). Two young adult males (S and W) were selected as normal subjects. Two patients (T and U) having cholecystitis associated with stones in the gallbladder but with normal liver function underwent cholecystectomy and a T-tube was placed for bile drainage. The patients received the test dose three days after surgery. Bile was then collected as previously described (8).

Samples consisting of 30–50 ml. of blood were collected in heparinized syringes 15, 30, 60, 120 and 240 minutes after the injection, and the plasma was immediately separated from the red blood cells. Urine and bile samples were collected 4, 12, 24 and 48 hours following the corticosterone administration. The method of hydrolysis and extraction of the various samples has been described in detail elsewhere (8). All extracts of plasma, red blood cells, urine and bile, as well as whole urine and whole bile were assayed for radioactivity in the manner previously described (8).

RESULTS

Approximately two months prior to 4-C^{14}-corticosterone administration, Subjects S and W had received a test dose of 4-C^{14}-cortisol, the results of which were reported in a preceding paper (8) regarding Subjects P and E, respectively. Plasma radioactivity following the injection of these two steroids in each of these control subjects was compared. The results are shown in Figures 1 and 2. It can be seen that the amount of radioactivity in the chloroform-soluble ("free") fraction of plasma fifteen minutes after the injection of 4-C^{14}-corticosterone is about half that present after 4-C^{14}-cortisol injection, and that thereafter the rate of disappearance was faster for the former compound. The amount of radioactivity in the plasma released by β-glucuronidase hydrolysis reached its peak earlier and was of greater magnitude following corticosterone administration than after cortisol. Minimal radioactivity was found in the red blood cells. The curves ob-

tained in the 2 patients with bile fistulas (Subjects T and U) were similar to those found in the normal subjects S and W (Fig. 3).

In Figure 4 the average levels of radioactivity in the free form and after β-glucuronidase hydrolysis following the injection of 4-C^{14}-corticosterone and 4-C^{14}-cortisol are plotted against time. This figure illustrates the much more rapid conversion of free corticosterone to conjugated metabolites.

The curves of the cumulative excretion of radioactivity in the bile of

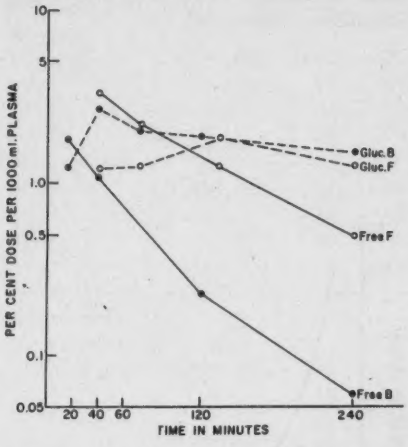


FIG. 1. Comparison of plasma radioactivity following injection of 4-C^{14}-corticosterone (black dots) and 4-C^{14}-cortisol (circles) in a control subject. (Solid line: "free" radioactivity. Broken line: radioactivity released after β-glucuronidase hydrolysis.)

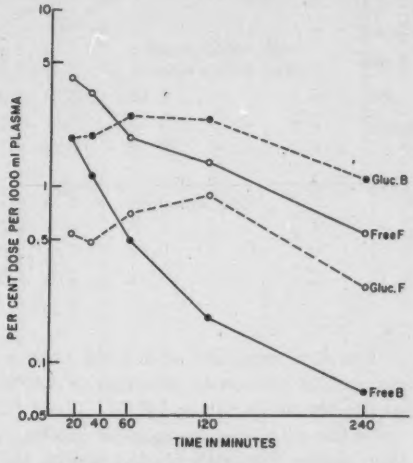


FIG. 2. Comparison of plasma radioactivity following injection of 4-C^{14}-corticosterone (black dots) and 4-C^{14}-cortisol (circles) in a control subject. (Solid line: "free" radioactivity. Broken line: radioactivity released after β-glucuronidase hydrolysis.)

Patients T and U are shown in Figure 5. They are compared with those obtained in Patients J and N of a previous study, who received 4-C^{14}-cortisol (8). Little or no isotopic activity was found in the free fraction. The distribution in the three other fractions was approximately similar to that found in urine, although the fraction released by strong acid hydrolysis was somewhat higher.

The data on the urinary excretion of radioactivity are shown in Table 1. In the 2 controls, 79 per cent of the injected dose was recovered during the forty-eight hours following administration of compound B. Less than 1 per cent was extractable without hydrolysis and over 30 per cent was extractable after β-glucuronidase hydrolysis. Following these two procedures, an additional 13 per cent of the dose was released after a 48-hour continuous

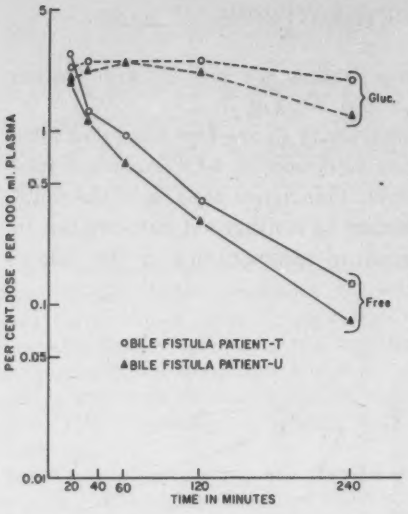
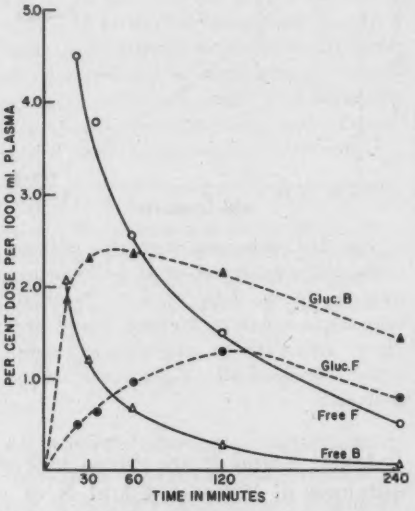


FIG. 3. Plasma radioactivity following administration of 4-C^{14}-corticosterone in Patients T and U with bile fistulas. (The solid lines represents the curves of chloroform-soluble steroids and the dotted lines those of steroids extractable after β-glucuronidase hydrolysis.)

FIG. 4. Comparison of average plasma radioactivity following injection of 4-C^{14}-corticosterone in Subjects S, W, T and U (solid line with open triangles for free fraction; dotted line with black triangles for the glucuronide fraction), and that following 4-C^{14}-cortisol in Subjects A, D, E, K and P of a previous study (8) (solid line with open circles for free fraction; dotted line with black circles for the glucuronide fraction).



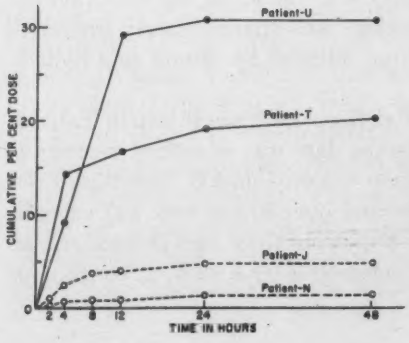


FIG. 5. Comparison of cumulative excretion of radioactivity in bile following administration of 4-C^{14}-corticosterone to fistula Patients U and T (black dots, solid line) and that following administration of 4-C^{14}-cortisol to Patients J and N in a previous study (8) (circles, broken line).

TABLE 1. CUMULATIVE URINARY EXCRETION OF RADIOACTIVITY FOLLOWING INJECTION OF 4-C^{14}-CORTICOSTERONE

Subjects	Injected dose (c.p.m.)	Hrs. after inject.	Per cent excreted in urine					
			Total	Free	β-Gluc.	Cont. extraction	Strong acid	Not accounted for
Normal subjects								
S	1,476,500	4	33.0	0.3	12.9	6.8	3.9	9.1
		12	59.0	0.5	22.1	10.	7.7	18.7
		24	70.0	0.7	27.5	11.5	9.2	21.1
		48	79.0	0.8	31.8	11.9	9.9	24.6
W	1,483,500	4	37.7	0.2	16.0	7.6	2.5	11.4
		12	57.6	0.4	23.2	11.	5.5	17.5
		24	70.0	0.5	30.0	12.8	6.9	19.8
		48	78.0	0.7	33.5	13.7	8.2	21.9
Bile-fistula patients								
T	1,390,000	4	28.8	0.4	13.3			
		12	43.0	0.5	17.7			
		24	56.0	0.7	21.7			
		48	70.8	0.9	24.3			
U	1,100,000	4	26.8	0.5	8.7	3.5	5.0	9.6
		12	47.4	0.6	16.8	5.8	8.3	16.5
		24	58.0	0.65	20.2	6.6	8.9	22.3
		48	63.1	0.75	22.0	6.7	9.3	25.1

extraction at pH 0.8. Subsequent hydrolysis with strong acid yielded 9 per cent as ether-soluble radioactivity. Approximately 23 per cent was unaccounted for in the various lipid-soluble fractions.

Although the total activity in the urine after forty-eight hours was significantly lower than in normal subjects after C^{14}-cortisol (approximately 90 per cent), the sum of biliary and urinary excretion in the bile fistula subjects was greater than 90 per cent; there seemed to be no significant difference between the 48-hour excretions of the metabolites of the two compounds when both routes were considered in each.

DISCUSSION

Since after the administration of C^{14}-corticosterone the radioactive "free steroids" disappear from the circulation faster, and the glucuronides appear

more rapidly, than after C^{14}-cortisol, the metabolism of corticosterone in the human being must be significantly more rapid. The concentration of radioactivity in these conjugates is also higher but the accumulation in the urine is no more rapid. These findings suggest that, even though corticosterone is rapidly metabolized, a smaller proportion of the conjugated products in the plasma is removed during passage through the kidney. Such a reduction must be due to a difference in the way the kidney handles the conjugated metabolites of corticosterone and cortisol.

Decreased excretion of corticosterone metabolites in the urine seems to be associated with increased secretion through the bile. The distribution of the "free" and conjugated compounds in this fluid is similar to that in the urine; apparently the two routes of excretion drain the same metabolic pool.

The fact that the urinary excretion of radioactivity by the bile-fistual patients was lower than that of the control subjects suggests that some entero-hepatic circulation was taking place, but the sum of urinary and bile radioactivity of the former, being higher than the isotopic activity in the urine alone of the normal individuals, demonstrated that the reabsorption of the steroid from the gut was incomplete.

The significance of the radioactivity extractable after removal of the "free fraction" and the "glucuronide fraction" remains somewhat obscure. As was pointed out previously (8), it is difficult to decide whether the continuous extraction at pH 0.8 was merely hydrolyzing glucuronides which had not been split by the enzymatic hydrolysis or whether it was hydrolyzing a group of conjugated compounds other than glucuronides. These remarks also hold for the radioactivity found after strong acid hydrolysis. Recently, Engel, Carter and Fielding (11) reported that, after the urine from a patient given corticosterone had been incubated with β-glucuronidase at 37° C. for three days, they were able to isolate three metabolites: pregnane-3α,11β,21-triol-20-one and its allopregnane isomer, and pregnane-3α,20α-diol-11-one. These metabolites account for 12.6 per cent of the administered corticosterone. The nature of the remaining metabolites, the forms in which they are conjugated and their relation to the radioactivity obtained after β-glucuronidase hydrolysis await investigation.

SUMMARY

The distribution of radioactivity in the blood, bile and urine after the injection of 4-C^{14}-corticosterone was studied as a function of time in 2 normal subjects and 2 patients with bile fistulas. The radioactivity of the chloroform-soluble ("free") steroid and the material which became chloroform-soluble after hydrolysis with β-glucuronidase, continuous extraction

at pH 0.8, and after boiling with 10 per cent hydrochloric acid, was determined in the samples.

The rate of disappearance of radioactivity in the "free" fraction from the plasma was markedly faster than that seen after 4-C^{14}-cortisol administration.

The radioactivity of the glucuronide fraction in plasma reached a maximum in thirty to sixty minutes. The peak was reached more rapidly and was of greater magnitude than that after 4-C^{14}-cortisol.

The bile contained from 20.5 to 31 per cent of the steroid. The data suggest that significant but incomplete entero-hepatic circulation was taking place.

Approximately three-fourths of the C^{14}-corticosterone was excreted in the urine in forty-eight hours. This is a smaller amount of isotopic activity than after 4-C^{14}-cortisol administration. The total excretion in the urine and bile following radioactive corticosterone equalled that of radioactive cortisol.

Even though the levels of conjugated steroid in the plasma during the initial four hours following 4-C^{14}-corticosterone administration were higher than those following 4-C^{14}-cortisol, the amount of radioactivity excreted in the urine during that time was similar following either compound. These data would seem to indicate that the conjugates of corticosterone are less readily excreted in the urine than are those of cortisol. The reverse was true for biliary excretion.

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METABOLIC EFFECTS IN MAN OF THE ACETIC-ACID ANALOGUES OF THYROXINE AND TRIIODOTHYRONINE

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TRIIODOTHYROACETIC acid (3:5-diiodo-4-(3′-iodo-4′-hydroxy-phenoxy)-phenyl acetic acid—hereinafter called triac) has been reported to cause, within thirty minutes, an increase in oxygen consumption in rat kidney slices (1). This effect was largely gone within four hours. In rats, triac and tetraiodothyroacetic acid (tetrac) have been tested for their antigoitrogenic properties and their effect on oxygen consumption. In general, both compounds are effective and have about one-tenth the potency of their respective parent compounds (2). It has been further shown that triac can induce changes in the feathers of birds, similar to those induced by thyroid hormone (3). In human subjects, triac has been reported to cause remission in the clinical features of myxedema and a depression in the serum cholesterol level, without change in basal oxygen consumption (4). In another study on a single case of myxedema a rise in basal metabolic rate (BMR) was observed after the administration of triac (5). It has been suggested that, because of its unusually rapid action *in vitro*, triac may represent the form in which the thyroid hormone exists in the tissues. (1). This is an attractive hypothesis biochemically, since the keto acids of both thyroxine (T_4) and triiodothyronine (T_3) have been identified, and further oxidation of the next lower acid (in this case, the acetic acid compound) seems a likely reaction (6). Furthermore, the presence of triac in the kidneys of rats given labeled T_3 has recently been demonstrated (7).

Triac and tetrac were studied in 2 patients with myxedema. In general, these compounds appeared to be qualitatively similar to the parent compounds (T_3 and T_4), although less potent.

PATIENTS AND METHODS

Patient A. S. was a 27-year-old male cretin who had been treated with desiccated thyroid since the age of 10 weeks. He had been first seen at Memorial Center in 1951, when exhaustive psychologic testing revealed an intelligence somewhat above normal.

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He had had no thyroid treatment for eight weeks at that time and had appeared myxedematous. His BMR had been −38 per cent, serum cholesterol level 398 mg. per 100 cc., thyroidal I^{131} uptake 0.8 per cent (counted over the neck), and serum protein-bound iodine (PBI) concentration 1.0 μg. per 100 cc. From 1951 until the present (1955) he had been treated with a variety of thyroid hormones. He had been taking 0.200 mg. of triiodothyronine (T_3) daily from April 1954 until six weeks prior to admission. On admission he had the signs and symptoms of hypothyroidism. His laboratory data are shown in Figures 1, 2, 3, 4 and 5.

Patient D. Y. was a 20-year-old girl with carcinoma of the thyroid, who had had a total thyroidectomy seven years previously. She had been maintained on desiccated thyroid, 90 mg. daily, until eight weeks prior to the present study. In addition, she had postoperative hypoparathyroidism and had been maintained on 50,000 units of vitamin D_2 daily for seven years. On admission she had the signs and symptoms of myxedema.

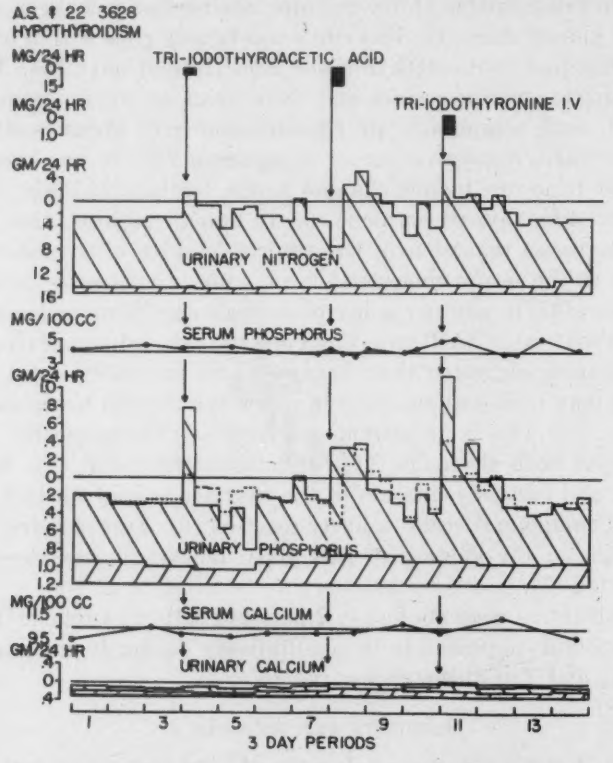

FIG. 1. Nitrogen and phosphorus balances in Subject A. S. (In this and succeeding figures, intake is plotted downward from zero and output is plotted upward. The fecal output is below that of the urinary output. The solid lines represent the experimental data. The dotted lines represent the theoretical values; for phosphorus this value was obtained from the nitrogen and calcium balances.)

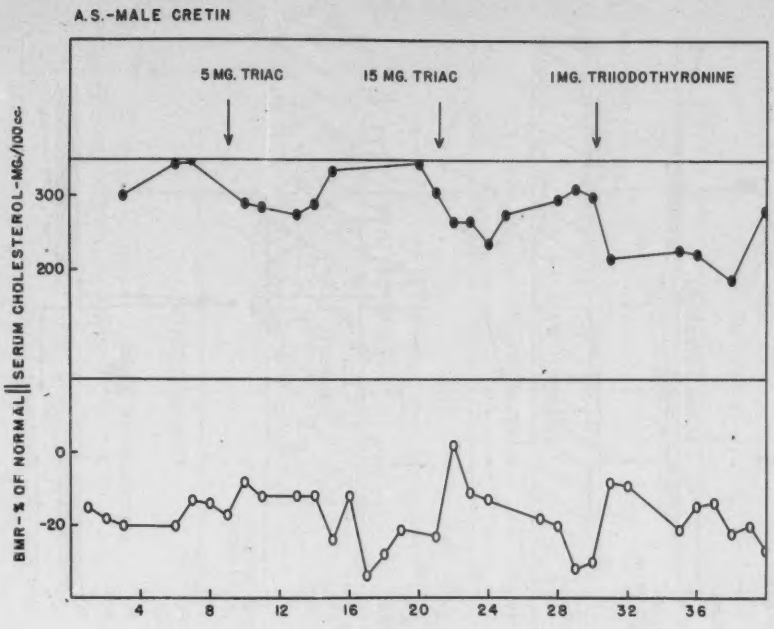

FIG. 2

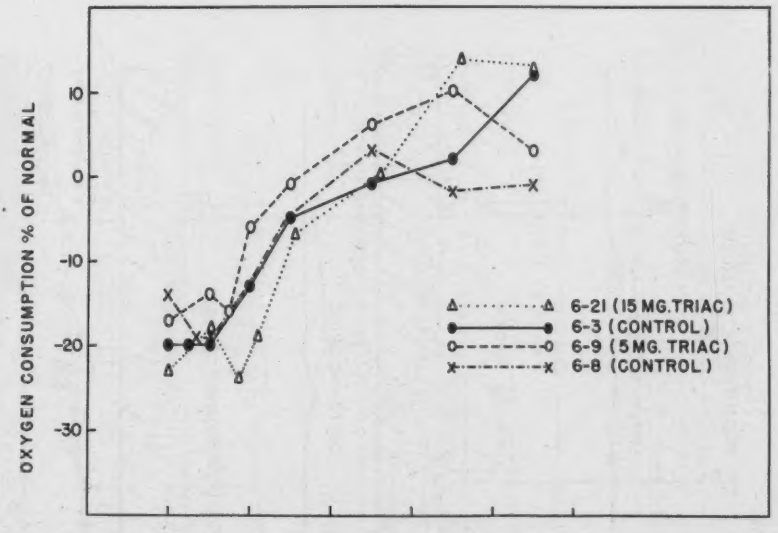

FIG. 3

FIG. 2. Subject A. S. BMR and serum cholesterol level. (The abscissa is plotted in days after admission to the hospital.)

FIG. 3. Oxygen consumption, as a percentage of normal BMR, determined at frequent intervals on four days. The dates are shown in the key to the graph. The doses of triac were given at 8:30 A.M.

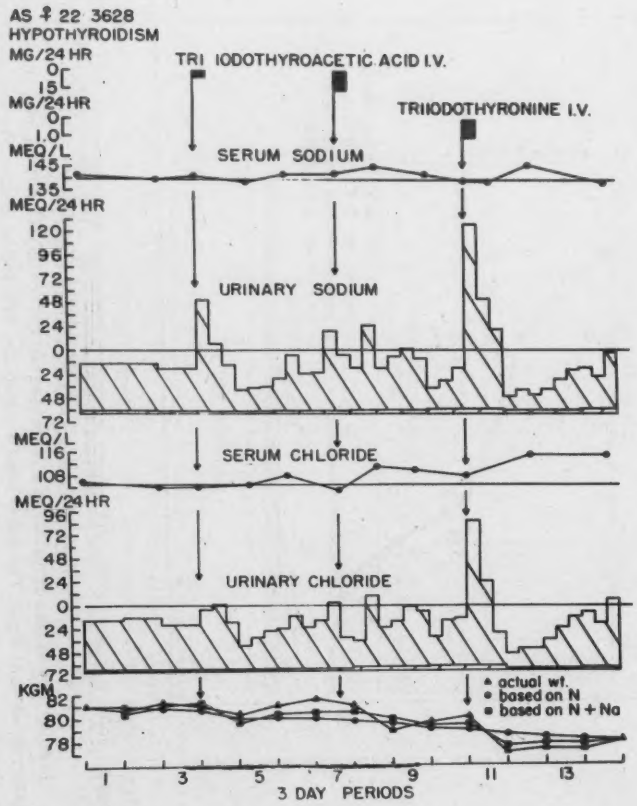

FIG. 4

FIG. 4. Subject A. S. Sodium and chloride balances.

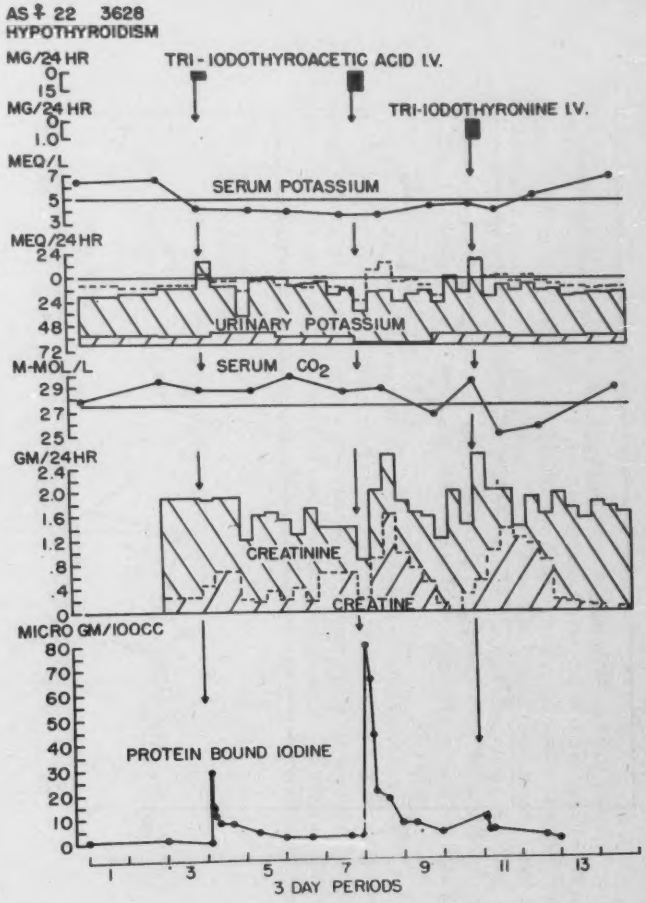

FIG. 5

FIG. 5. Potassium balance and CO_2, creatinine, creatine and PBI data.

Fig. 4. Subject A. S. Sodium and chloride balances.

Fig. 5. Potassium balance and CO_2, creatinine, creatine and PBI data.

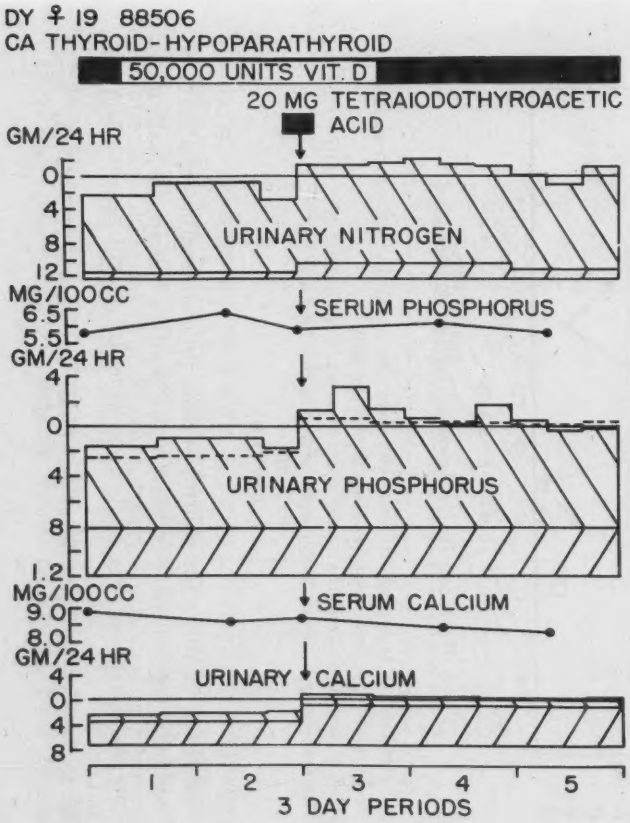

Fig. 6

Fig. 6. Subject D. Y. Nitrogen and phosphorus balances.

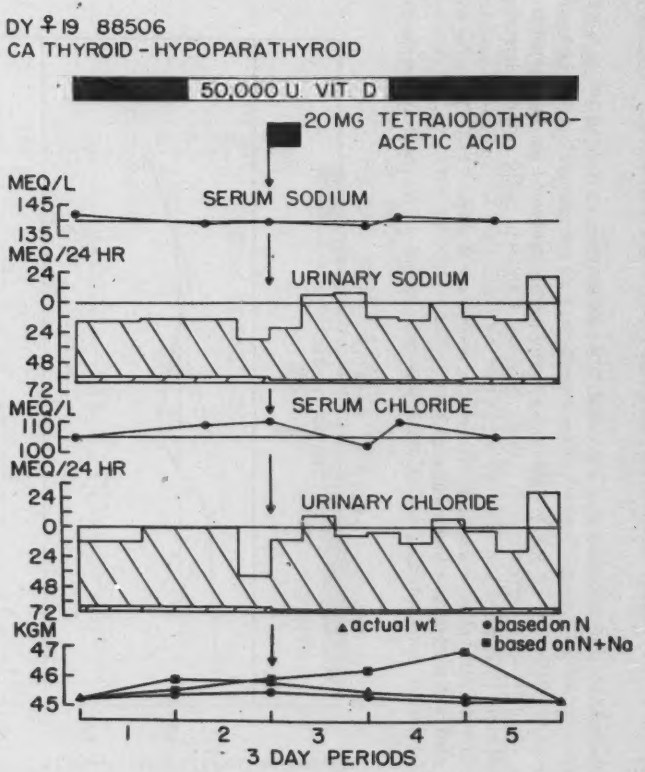

Fig. 7

Fig. 7. Sodium and chloride balances.

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The laboratory data are shown in Figures 6, 7, 8 and 9. A tracer dose of I^{131} showed 3 per cent uptake in the neck and a urinary excretion of 85 per cent in forty-eight hours.

Both patients were on the metabolic ward and were maintained on daily repetitive diets calculated to keep them in caloric balance. Complete daily urine collections, pooled stool collections and aliquots of the diet were analyzed for sodium, potassium, chlorine, calcium, phosphorus and nitrogen, as previously described (8). The urine was also analyzed for creatine and creatinine.

The serum protein-bound iodine level was determined at frequent intervals (9).

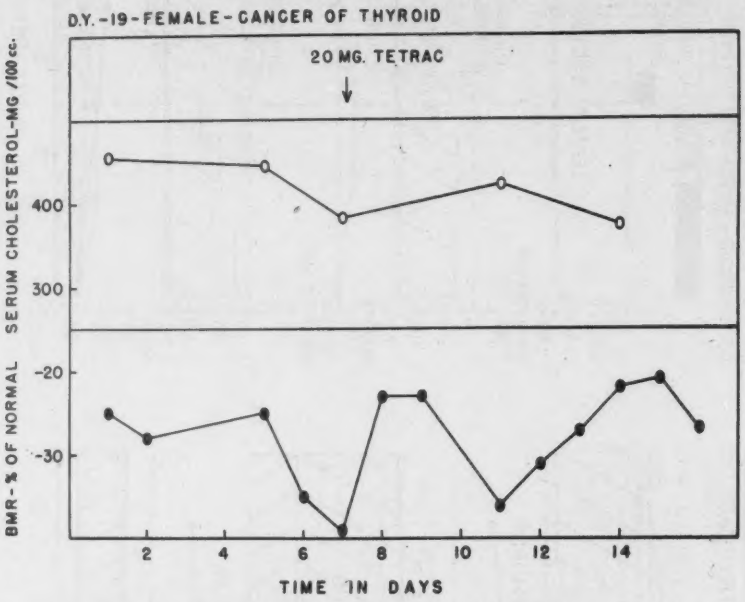

FIG. 8. BMR and serum cholesterol level in Subject D. Y.

A basal metabolism test was performed six days per week, and oxygen consumption was measured at frequent intervals throughout the day.

Triac[1], tetrac[1] and triiodothyronine[2] (T_3) were made up in 0.02 N NaOH in 0.9 per cent NaCl and given intravenously as indicated in the figures.

RESULTS

Subject A. S.

Triac (5 mg.). During the control periods prior to treatment, while A. S. was becoming myxedematous, there was a positive balance for both nitro-

[1] Triac and tetrac were very kindly given us by Dr. R. Pitt-Rivers of Mill Hill, England.

[2] Kindly supplied by Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

gen and phosphorus. Triac in a dosage of 5 mg. caused a definite loss of nitrogen and phosphorus and an actual negative balance (Fig. 1).

The maximal effect appeared to be on day 1 or 2. This was also true for the urinary creatine, but the urinary creatinine did not change significantly. There was a slight rise in BMR (Fig. 2) but the daily variation during the control periods makes assessment of its magnitude difficult.

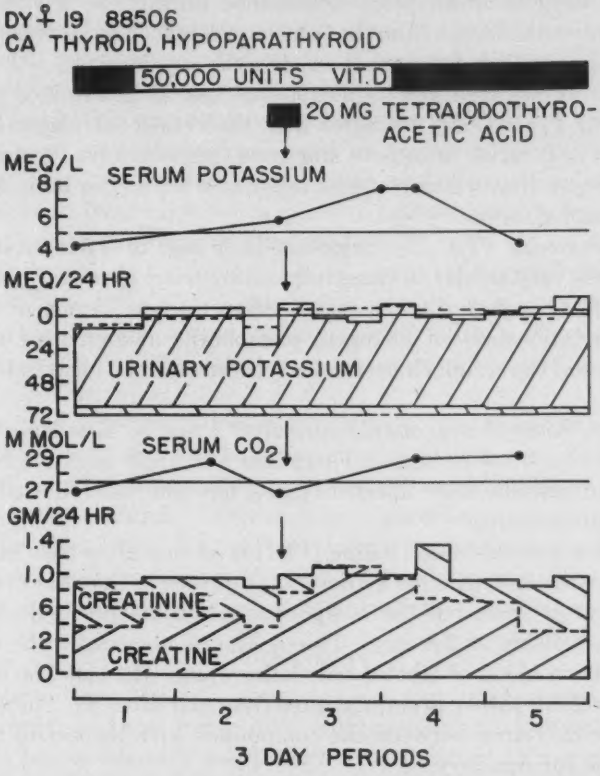

FIG. 9. Potassium balance, and CO_2, creatinine and creatine data in Subject D. Y.

The oxygen consumption during the day of injection (5 mg. of triac) did not differ significantly from oxygen consumption on control days (Fig. 3). There appeared to be a small loss of both sodium and chloride lasting two days after this injection (Fig. 4). Afterwards, a period of rather marked retention of both sodium and chloride occurred. No significant changes in potassium excretion were observed (Fig. 5).

There was a positive calcium balance prior to treatment. There appeared

to be definite increases in the urinary excretion of calcium after both doses of triac, but these were of such a small magnitude that their significance is not clear (Fig. 1).

Triac (15 mg.). After 15 mg. of triac the effects were more pronounced. In particular, the BMR rose to −2 per cent. The rise was more impressive if one considers the effect of triac on the BMR during the four previous days. The oxygen consumption determined during the day of injection was probably no different from that on control days. The changes in sodium and chloride were less clear-cut than after the smaller dose.

The ratio of nitrogen loss to phosphorus loss, as determined previously with T_3 and T_4 (10), did not agree with theoretical calculations based on N/P and Ca/P ratios in muscle and bone, respectively. Phosphorus was lost in excess of that which could be accounted for on the basis of nitrogen and calcium balances.

Triiodothyronine (T_3). The injection of 1 mg. of T_3 induced changes qualitatively very similar to those induced by triac. However, the response to 1 mg. of T_3 appeared to be greater than that to 15 mg. of triac. The temporal relationships of nitrogen, phosphorus and creatine excretions, the BMR, and the serum cholesterol level seemed to be identical with those for triac.

Comment. After 15 mg. of triac and after 1 mg. of T_3 a definite increase in urinary creatinine occurred. There was a definite negative balance for sodium and chloride after injection of T_3, but this was followed by retention of both elements.

The serum protein-bound iodine (PBI) level rose after triac and after T_3 (Fig. 5). It was not possible to determine precisely the rate of fall of the PBI level after triac, but the component which disappeared most slowly fell with a half-life of 2.4 days. This is almost identically the disappearance rate from blood of labeled triiodothyronine (10, 11). The serum cholesterol level fell rather promptly after triac and after T_3. There appeared to be little difference between the compounds with respect to rapidity of fall or time for recovery.

No significant changes were observed in the levels of serum phosphorus, sodium, or chloride. The mild hyperkaliemia prior to treatment is unexplained. Clinically there were few changes after injection of 5 mg. of triac. After 15 mg. of triac there was an increase in pulse rate and a mild, transient tremor of the hands. The patient also noted mild "jitteriness" for one day. After 1 mg. of T_3 the tachycardia was more pronounced and a real increase in perspiration occurred. Manual tremor and nervousness were also observed. There was some peeling of the skin of the hands after 15 mg. of triac, and this persisted for three or four weeks.

A definite weight loss occurred after all three injections. As may be seen in Figure 4, this was fairly well accounted for on the basis of sodium and nitrogen loss.

Subject D.Y.

Tetrac was given in a single intravenous dose of 20 mg. to Subject D.Y. The results were similar to those seen in A.S. after injection of triac or T_3, but required longer for a maximum effect and seemed more prolonged. The nitrogen loss was maximal on day 4 and lasted seven days (Fig. 6). The phosphorus loss was maximal on day 2 and lasted at least seven days. As with T_3 and triac, and as previously noted with T_4, there was a slightly greater loss of phosphorus than could be accounted for on the basis of nitrogen and calcium balances. There was a real change in calcium metabolism, mainly in fecal excretion; a positive balance prior to tetrac changed to a slightly negative balance afterward.

There appeared to be a slight loss of both sodium and chloride after injection of tetrac (Fig. 7). No significant changes were seen in potassium excretion (Fig. 9). The serum cholesterol level fell to a degree which was probably significant. The BMR was rather variable and there was no clear-cut rise (Fig. 8).

The creatine excretion followed the nitrogen loss, reaching a maximum in three days and lasting seven days (Fig. 9). The serum PBI level rose after injection of tetrac to a 170 μg. per 100 cc., and fell after seven hours to 112 μg. per 100 cc. Further determinations were not made.

Clinically there were very minor changes in heat tolerance and skin temperature. A slight tachycardia was noted. In general, the subjective symptoms and the physical signs were noteworthy for their absence.

DISCUSSION

These data show that, in general, triac and tetrac cause metabolic changes similar to those induced by thyroxine or triiodothyronine. Triac appears to be considerably less potent than T_3, since 15 mg. of triac had effects comparable to those seen after injection 1 mg. of T_3 in the same patient. Qualitatively the effects were almost identical. The time sequence of changes with triac and with T_3 also were indistinguishable. In particular, no abrupt and transitory alterations were noted. Triac caused changes in the BMR which appeared to be related to such other parameters of effect as loss of nitrogen or decrease in the cholesterol level, as observed after administration of T_3. Different dosages and routes of administration prevent comparison with the data of an earlier report (4). The reason for the marked phosphorus diuresis is not clear. The absence of change in the level

of serum phosphorus militates against a primary renal effect. Since clearance studies were not carried out, this possibility is not completely eliminated. The source of the phosphorus is also not clear. Some of it undoubtedly accompanied nitrogen, and it may be that the protein of myxedema fluid has an unusually high P/N ratio. It is also possible that a fall in the phospholipid level, such as usually accompanies a fall in the cholesterol level (12), released additional phosphorus. Finally, the phosphorus may have come from muscle organic phosphate, more or less accompanying creatine. We calculated the total nitrogen loss and the ratio of N/P lost following administration of the various doses of the drugs. Since the patients were in a state of positive nitrogen and phosphorus balance prior to receiving these compounds, the amount excreted above initial figures was used. This was more than the actual negative balance. The N/P ratio was not calculable in the case of the 5-mg. dose of triac, because there was (for the six-day period of the study) no change in phosphorus excretion over control values. With this exception, the N/P ratio was fairly constant.

The effect of thyroactive materials on calcium excretion is not entirely clear. Earlier studies on hyperthyroid and hypothyroid patients seemed to show that the thyroid hormone increases the rate of calcium excretion (13). More recently, in acute experiments, no effect was noted (10, 14). In these experiments there was at least a suggestion that calcium excretion was increased by thyroid hormone.

The effect of thyroid substance on sodium, chloride, and potassium metabolism is also unclear. Some investigators have found marked effects in both normal and myxedematous subjects (15), whereas others have found none (10, 14, 16). The present studies do not appear to clarify the problem, because a rather clear-cut loss of both sodium and chloride followed by a positive balance, observed after 5 mg. of triac, was not seen after a larger dose of the same drug. It is possible that sodium loss is a second-order effect and is due to changes in the levels of aldosterone or hydrocortisone which could be initiated by very large doses of thyroactive materials. Sodium diuresis did not appear to be secondary to changes in glomerular filtration, since it took place following injection of 5 mg. of triac although there was no change in urinary creatinine. On the other hand, after injection of T_3, marked sodium loss and an increase in urinary creatinine occurred. Urinary creatinine is assumed to be a measure of glomerular filtration rate.

The rise and fall in the serum PBI level after administration of these drugs is of interest. In a general way, triac was metabolized at a rate that very closely approximated that for T_3. The final volume of distribution of triac was 340 cc. per Kg. This is considerably less than that of T_3, and errors

in estimation of PBI concentration seem insufficient to account for this difference (11). In view of the marked difference between T_3 and triac with regard to binding by thyroxine-binding protein, the similarity of their rates of disappearance from the blood is somewhat surprising (17). In this myxedematous patient, triac appeared to be somewhat less than one-fifteenth as potent as T_3, but was otherwise indistinguishable from T_3 in its effects. Tetrac was less potent than T_4, but otherwise its action was indistinguishable from that of T_4. These rather inexact estimates of potency agree fairly well with those reported for the rat (2).

SUMMARY

Triiodothyroacetic acid (triac) and tetraiodothyroacetic acid (tetrac) have been studied in 2 patients with myxedema. The effects on oxygen consumption, serum cholesterol level, urinary creatine, and nitrogen and phosphorus balances were similar to those of triiodothyronine and thyroxine, but triac and tetrac were considerably less potent.

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INHIBITION OF THYROIDAL RADIOIODINE UPTAKE FOLLOWING INTRAVENOUS ADMINISTRATION OF THYROXINE TO NORMAL AND TO HYPOPHYSECTOMIZED ADULT SUBJECTS*

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IT HAS been demonstrated in animals (1) and in man (2) that continued administration of thyroid substance diminishes thyroidal activity. Farquharson and Squires (2) showed that after cessation of thyroid hormone ingestion in human subjects, there was a reduction in metabolic rate to a level below that observed initially. This finding was confirmed by Riggs and his associates (3) who noted, in addition, that withdrawal of desiccated thyroid was followed by a decrease in the level of serum precipitable iodine and in metabolic rate which persisted for several weeks. These observations indicated that exogenously administered thyroid hormone has a depressive effect on the function of the thyroid gland, and that this effect is manifested beyond the period of hormone administration.

Demonstration of an inhibition of thyroidal activity in man *during* administration of thyroid hormone had to await the development of a method of measurement of endogenous thyroidal function not obscured by the action of the exogenous thyroid substance. Since the advent of techniques for determining radioiodine uptake by the thyroid gland, it has been shown that thyroidal iodine-accumulating ability is reduced in the presence of various thyroid preparations administered by the oral route to euthyroid subjects (4–9). The observation that desiccated thyroid in physiologic amounts inhibited a measurable function of the thyroid gland (5) strengthened the belief that thyroidal activity might be held in check normally by the level of endogenous hormone. In accord with the concept of pituitary-thyroid interrelationship (10), the hypothesis was advanced that exogenous

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thyroid hormone diminished thyroidal iodine-accumulating capacity by reducing thyrotropin activity. Moreover, it was suggested that the amount of endogenous thyroid hormone produced in the euthyroid subject was represented by the quantity of administered thyroid substance required to exert maximal inhibition of radioiodine uptake (5).

Other investigations delineated the effects of exogenously administered thyrotropin on thyroidal accumulation of radioiodine in normal subjects, before and during the administration of desiccated thyroid (4, 6). Although it was shown that stimulation with thyrotropin overcame thyroxine-induced depression, the total thyroidal collection of radioiodine failed to reach the pre-thyroxine levels (6). This was interpreted as suggesting that administered thyroid substance acted primarily by inhibiting thyrotropin activity, but that it also exerted a minor depressant effect directly on the thyroid gland.

These observations led us to seek confirmation of the role of thyrotropin in the thyroidal depression induced by thyroid hormone. It was considered that if the inhibition of thyroidal function be mediated by suppression of endogenous thyrotropin, then the pattern of decline in thyroidal radioiodine uptake induced by thyroxine should be similar to that known to occur after hypophysectomy (11, 12). Moreover, if the inhibition results solely from reduced thyrotropin activity, then administration of thyroid hormone to hypophysectomized subjects should result in no further diminution of the uptake. Finally, replacement of the endogenous quantity of thyrotropin in such individuals should restore uptake to normal, even in the presence of exogenous thyroxine.

The present study is concerned with the effect of thyroxine, administered in a single intravenous injection, on thyroidal radioiodine accumulation in 33 euthyroid subjects and in 2 hypophysectomized patients. In 1 of the latter the response of thyroidal uptake to injected thyrotropin was measured before and after thyroxine administration.

PROCEDURE

Thirty-three apparently euthyroid subjects were selected for serial radioiodine uptake tests which were performed before, and at varying intervals following, intravenous injection of thyroxine. The group consisted of 1 white and 18 colored females, and 7 white and 7 colored males; age extremes were 15 and 59 years. Most subjects were patients on the medical wards convalescing from a variety of illnesses; none presented clinical evidence of endocrine disorder. In each, a thyroidal radioiodine uptake test performed prior to thyroxine administration served to confirm the clinical impression of euthyroidism. Factors known to influence radioiodine uptake were excluded insofar as possible prior to the study. No clinical changes developed which could be attributed to thyroxine or radioiodine administration.

Radioiodine (I^{131}) without added carrier iodide was administered by the oral route in a dosage of 50 microcuries in 20 ml. of tap water, followed by a single rinse of the

container. Serial counts were made at specified intervals using an end-window Geiger-Müller tube held in contact with the skin overlying the thyroid gland. At each reading, duplicate one-minute counts were made over the thyroid area, and the values were averaged. The principle of "accumulation gradient" described by Astwood and Stanley (13) was used in the recording of data. The counts per second per 100-microcurie dose obtained over the thyroid area were plotted (ordinate) against the square root of the elapsed time in minutes (abscissa) after ingestion of radioiodine. The rate of uptake was expressed in terms of the slope of the straight line which resulted, and was determined by calculating the ratio of ordinate/abscissa at the eighth hour of accumulation. The range of slope values in normal subjects in this laboratory has been established at 0.5 to 1.5.

Part I

In Part I of this study, the alteration in thyroidal uptake of radioiodine immediately after thyroxine administration was investigated. Readings were obtained at intervals of fifteen to thirty minutes after ingestion of radioiodine, until linearity of the plotted values was established, following which thyroxine was injected and readings continued at the same frequency through the sixth or eighth hour. At twenty-four hours, readings were uniformly obtained.

Part II

In Part II, the delayed effect of thyroxine on thyroidal radioiodine accumulation was studied. In each test, the linear curve was plotted from readings recorded at the first, sixth and twenty-fourth hour after ingestion of I^{131}, and the slope value derived. In each repetition of the test, measurement of residual radioactivity over the thyroid area was made immediately before the dose of radioiodine was given, and this value was appropriately subtracted from subsequent readings. In each subject, from 1 to 4 uptake tests were performed at varying intervals following thyroxine administration; slope values were determined and compared with pre-treatment values. In 3 subjects, protein-bound iodine levels[1] were obtained before and at intervals after thyroxine administration.

Radioiodine uptake tests were carried out before and after thyroxine administration in 2 patients in whom hypophysectomy had been performed for therapy of osseous metastases from primary carcinoma of the breast.[2] In 1 of these patients, the effect of thyroxine on thyroidal uptake following stimulation by exogenous thyrotropin was investigated; on 2 occasions a single 25.0-mg. dose of thyrotropin[3] was injected by the intramuscular route.

In each subject a single dose of thyroxine (1 mg. per ml. of solution) was administered slowly into an antecubital vein. In Part I of the study, *dl*-thyroxine[4] was given to each subject. In Part II, 19 subjects received *dl*-thyroxine[5] and 4 were given *l*-thyroxine.[6]

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[1] Analyses made by Bio-Science Laboratories, Los Angeles, California.

[2] Permission to perform the studies was kindly granted by the patients and by their private physician, Dr. William G. Speed, III.

Kindly supplied by:

[3] The Armour Laboratories.

[4] Roche-Organon, Inc.

[5] E. R. Squibb & Sons.

[6] Smith, Kline and French Laboratories.

The hypophysectomized patients received *l*-thyroxine. Each thyroxine compound was obtained in crystalline form and was prepared in lots of 20 mg. per 20 ml. of distilled water, with two drops of 1N sodium hydroxide added to effect solution. The solutions were autoclaved at 15 pounds pressure per square inch for twenty minutes and allowed to cool prior to injection.

RESULTS

Part I: *Immediate effect of thyroxine on thyroidal radioiodine uptake*

In Table 1 a summary is presented of the effects of administering *dl*-thyroxine in single doses varying between 0.75 and 5.0 mg. to 10 subjects

TABLE 1. IMMEDIATE EFFECT OF INTRAVENOUSLY ADMINISTERED THYROXINE ON THYROIDAL I^{131} ACCUMULATION IN EUTHYROID SUBJECTS

Subject	Dose of thyroxine (mg.)	Inhibition of uptake			Validity of response
		Hrs. after I^{131}	Hrs. after thyroxine	Duration in hrs.	
W.J.	4.0	2½	¾	2½	Definite
T.F.	0.75	2¼	¾	2¼	Definite
G.P.	3.0	2½	½	1½	Definite
M.F.	2.0	3	1½	1	Definite
H.M.E.	4.0	4¾	2¾	1½	Questionable*
B.M.W.	1.0	2¾	¾	1¼	Questionable†
C.M.	5.0	4¼	2¼	2¼	Questionable‡
W.W.	2.0		No effect		
W.G.	2.8		No effect		
W.C.	4.0		No effect		

* Inhibition late in course of uptake test.

† Pre-thyroxine curve not linear, due to slight scatter of readings.

‡ Inhibition late in course of tracer test; scatter of late readings.

within two hours following ingestion of radioiodine. In 4 subjects, further thyroidal accumulation of radioiodine abruptly ceased within half an hour to one and a half hours after injection of thyroxine. This suppressive effect persisted for a period lasting from one hour to two and a half hours, and was immediately followed by a resumption of uptake at approximately the original rate. In 3 subjects this effect was slight, and in 3 others no effect was demonstrated. No relation was shown between the degree of inhibition and either the amount of thyroxine administered or the value of the control slope.

Serum inorganic iodide levels obtained in 1 subject (B.M.W.) immediately prior to injection of thyroxine and during the phase of inhibition of uptake revealed no change (3.2 and 3.1 micrograms per 100 ml., respec-

tively), but the suppressive effect was slight. To test further the possibility that the inhibitory effect might be due to associated or liberated inorganic iodide, uptake tests were performed similarly in 3 other euthyroid subjects in whom only potassium iodide was given intravenously in a quantity of 3.4 mg., which is the iodine-equivalent of 4.0 mg. of thyroxine. Two subjects exhibited complete cessation of uptake within fifteen minutes after injection, and this condition persisted during the ensuing five and a half hours of observation. In the third subject, there was no effect.

Part II: *Delayed effect of thyroxine on thyroidal radioiodine uptake*

A single injection of 3.0 mg. of *dl*- or *l*-thyroxine was administered to each of 22 subjects. A twenty-third subject (J.W.) was given only 1.5 mg. of *dl*-thyroxine because of the appearance of transient sinus tachycardia during the injection. Tests of radioiodine uptake were carried out at intervals varying between four hours and forty days after administration of thyroxine. In Table 2 the slope values of thyroidal uptake in each test are presented.

In 21 of the 23 subjects, there was a significant diminution below the control level of thyroidal radioiodine accumulation at one or more intervals tested. Among these 21 subjects, the inhibitory effect was less uniform in the earlier intervals. Data in Table 2 show depression of uptake in 1 of 4 subjects studied at the four-hour period, and in 16 of the remaining 17 subjects when first tested at later time-periods. This effect was particularly consistent at the seven-day and fourteen-day intervals. Of the 21 determinations in the responsive subjects at the latter time-periods, 18 were decreased below control levels.

Comparison between initial and later tests (Table 2) reveals that suppression of thyroidal uptake was more marked in degree in the later time-periods. Of 12 slope values which were decreased below the control level at the seven-day to nine-day period in the repeated tests, only 2 had been more markedly depressed at an earlier time period. This trend is clearly demonstrated in Figure 1, which shows the comparative alteration in thyroidal uptake following thyroxine administration. Of 10 diminished slope values determined between four hours and two days after thyroxine, only 2 represented decrements of a magnitude greater than 60 per cent of the control. By contrast, of 25 reduced slope values determined from three through fourteen days after thyroxine, 21 represented a depression of this order. The decline in 8 of the latter amounted to 80 per cent or more of the control value, but only 1 was lowered to zero level. Although thyroidal suppression was marked within four days after injection of thyroxine, attainment of the maximal observed range of depression in a consistent manner was not demonstrated until the seven-day to nine-day interval. In

TABLE 2. DELAYED EFFECT OF THYROXINE ADMINISTERED INTRAVENOUSLY ON THYROIDAL RADIOIODINE ACCUMULATION IN EUTHYROID SUBJECTS

Subject*	Slope values of I^{131} uptake								
	Before thyroxine	At various times after administration of thyroxine							
		4 hrs.	1 day	2 days	3 days	4 days	7–9 days	11–14 days	18–40 days
L.H.	1.52	1.99					0.29		
I.P.	1.20	0.73					0.05		
J.K.	1.18	1.35					0.22		0.82 (40 days)
M.A.	0.85	0.93					0.18		0.98 (31 days)
C.S.	0.81		0.27				0.09		
S.C.	0.77		0.18				0.11		
B.M.H.	0.68		0.76				0.22		
B.H.	0.66		0.39						
M.Y.	0.59		0.48				0.16		
G.G.	0.47		0.27				0.46		
O.A.	0.76			0.53			0.69	0.98	
J.W.†	0.44			0.31			0.13	0.17	
G.F.‡	0.43			0.35			0.16		
P.C.‡	0.70			0.43			0.25		
M.E.	0.65				0.62		0.75		0.86 (18 days)
E.S.	0.50				0.00		0.13		0.37 (18 days)
B.W.	1.24					0.47		0.20	0.51 (25 days)
H.D.	0.93					0.24		0.31	
M.D.	0.62					0.25		0.22	0.07 (25 days)
F.W.	0.50					0.50		0.69	
B.C.	0.55					0.29			
A.N.‡	0.96						0.18		
J.G.‡	0.55						0.32		

* All subjects received 3.0 mg. of *dl*-thyroxine, except as noted.

† Received 1.5 mg. of *dl*-thyroxine.

‡ Received 3.0 mg. of *l*-thyroxine.

most subjects, the inhibitory effect persisted two weeks after administration of the thyroxine, and thereafter lessened.

The general pattern of declining thyroidal radioiodine collection consisted of an effect which was variable in degree at one day, moderate at four days, and marked at seven days, followed by pronounced inhibition at fourteen days after thyroxine injection. Occasional exceptions to this trend were noted. The most striking example was the absence of any suppressive effect on the uptake in 2 subjects (M. E. and F. W.) studied at the usual intervals. In contrast, 1 subject (E. S.) demonstrated inhibition to the zero level on the third day after injection. Two others (C. S. and S. C.),

within one day after injection of thyroxine, had as marked a degree of depression as that occurring in most subjects at seven days.

In order to exclude the possibility that administration of several tracer doses of radioiodine might alter the capability of the thyroid to accumulate radioiodine, 2 euthyroid subjects underwent several uptake studies. Variations in slope value did not exceed 0.1—a change not considered significant

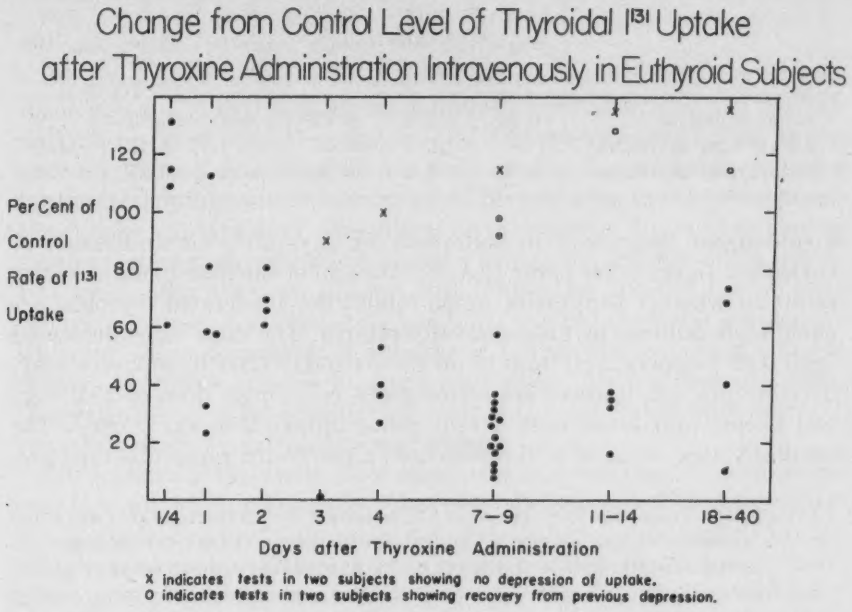

FIGURE 1

in view of the marked alterations in slope usually observed following injection of thyroxine.

No correlation was apparent between the height of the control slope value and any component of the depressive effect. There was no significant difference in thyroidal suppression between subjects given *l*-thyroxine and those given *dl*-thyroxine. In 3, serial protein-bound iodine determinations demonstrated that the increased serum levels resulting from injection of 3.0 mg. of *l*-thyroxine had declined toward normal at a time when thyroidal uptake was markedly suppressed (Table 3).

The effect of an intravenous injection of 3.0 mg. of *l*-thyroxine on the thyroidal uptake of radioiodine was observed in each of 2 hypophysectomized patients (Table 4). In one (C. D) the slope value (0.25) obtained on the fifth day after hypophysectomy was essentially unchanged (0.18) on

TABLE 3. RELATION BETWEEN SERUM PROTEIN-BOUND IODINE LEVELS AND SLOPE VALUES OF I^{131} UPTAKE AT VARIOUS TIMES AFTER ADMINISTRATION OF 3.0 MG. OF *l*-THYROXINE INTRAVENOUSLY IN EUTHYROID SUBJECTS

Time	Subject					
	J.G.		G.F.		P.C.	
	Slope	PBI (μg./100 ml.)	Slope	PBI (μg./100 ml.)	Slope	PBI (μg./100 ml.)
Before thyroxine	0.55	5.3	0.43	4.8	0.70	7.3
2 Days after thyroxine	—	10.2	0.35	13.4	0.43	14.0
7 Days after thyroxine	0.32	5.3	0.16	8.0	0.25	9.3

a subsequent determination performed six days after administration of thyroxine. In the other patient (A. Y.) the aim of the investigation was to ascertain whether *l*-thyroxine would inhibit the accelerated thyroidal accumulation induced by exogenous thyrotropin. The slope value decreased from 0.62 preoperatively to 0.15 on the sixth day after hypophysectomy. Thyrotropin was injected intramuscularly in a single dose of 25.0 mg., and twenty-four hours later a radioiodine uptake test was started. The resultant slope value of 0.50 represented a rise to the range observed pre-

TABLE 4. EFFECT OF ADMINISTRATION OF THYROTROPIN ON THYROIDAL ACCUMULATION OF RADIOIODINE AND ON SERUM PROTEIN-BOUND IODINE LEVELS BEFORE AND AFTER ADMINISTRATION OF THYROXINE IN HYPOPHYSECTOMIZED PATIENT

Date (1954)	Procedure	Slope value of I^{131} uptake	Serum PBI (μg./100 ml.)
Aug. 12		0.62	
14*	Hypophysectomy		
20		0.15	4.5
22	Thyrotropin†		
23		0.50	
24	Thyroxine‡		4.0§
29	Thyrotropin†		4.7§
30		0.61	5.8

* Patient received daily cortisone, 200 mg., from August 14 to 17; 100 mg. from August 17 to 19; and 50 mg. thereafter.

† Thytropar (Armour Laboratories), 25.0 mg., injected in a single dose intramuscularly.

‡ *l*-Thyroxine, 3.0 mg., injected in a single dose intravenously.

§ Obtained immediately prior to injection.

operatively. Thyroxine was then administered and the thyroidal response to thyrotropin re-examined six days later in an identical manner. The resultant slope value of 0.61 was slightly higher than that observed after the initial administration of thyrotropin. Serum protein-bound iodine levels were within normal limits; of interest was the similarity in levels before and five days after injection of thyroxine, and the slight increase following stimulation with thyrotropin.

DISCUSSION

In demonstrating that physiologic amounts of exogenous thyroid hormone suppressed thyroidal accumulation of radioiodine in normal individuals, Greer (5) clarified the temporal and dosage factors concerned. Ingestion of 3 grains of desiccated thyroid daily resulted in a moderate reduction in thyroidal uptake within four days, and in depression to the "myxedematous" range in eight days. The inhibition persisted at this level as long as administration of thyroid was continued, and ceased entirely within one or two weeks after the hormone was withdrawn. One third of the subjects receiving 1 grain of thyroid daily exhibited marked thyroidal depression; when the dose was raised in increments of 1 grain, the remaining subjects showed a stepwise increase in suppressive effect. After depression to the "myxedematous" level was effected, further increase in the daily dose did not result in additional inhibition of uptake.

The findings of this study show agreement in major respects with previous observations. Administration of a relatively large quantity of thyroxine in a single dose results in thyroidal depression which is only slightly earlier in appearance and no more intense in magnitude than that following daily ingestion of physiologic amounts of desiccated thyroid. The similarity between the thyroidal inhibitions observed following administration of thyroid hormone by the oral and by the intravenous route necessitates reconsideration of previous conclusions. It seems reasonable to consider that suppression resulting from daily ingestion of hormone may be effected entirely by the first few doses given. Based on this premise, determination of the daily amount of exogenous thyroid substance necessary for maximal inhibition of thyroid function (5) permits no conclusion as to the quantity of hormone actually required for this effect. The relatively small quantitative difference between effective and ineffective daily doses of desiccated thyroid suggests that a critical amount of hormone must be administered within a definite unit of time in order to initiate maximal inhibition.

The delay in the development of maximal thyroidal depression following intravenous injection of hormone establishes the existence of a true lag-period, independent of factors of absorption and accumulation. This implies that the delay lies in initiation or operation of the thyroid-suppressing

mechanism. Inasmuch as variable degrees of inhibition occur within one day after thyroxine administration, it appears likely that activation of the system is prompt and that time is required for full effects of its operation to become manifest. Accumulated observations indicate that the functioning of the thyroidal-suppressing mechanism is (a) initiated by variable amounts of thyroid hormone in different subjects, (b) independent of the serum protein-bound iodine level (14, 15), (c) related in degree to graded quantities of hormone, and (d) followed by a gradual decline of thyroidal radioiodine-accumulating ability. The interpretation of this mechanism on a physiologic basis merits further discussion.

Several studies have shown that administration of thyroid diminishes the response of the thyroid gland to thyrotropic hormone. Loeb and his associates (16) found in the guinea pig that thyroid substance inconsistently lessened the hypertrophy of the thyroid gland induced by pituitary extract. In intact guinea pigs and in hypophysectomized rats, the increase of acinar-cell height in thyroid epithelium following administration of thyrotropin appeared to be reduced by thyroxine, as was radioiodine accumulation in hypophysectomized rats (1). The validity of transferring these observations to man is not known. It has been generally believed that the depressive effect exerted by thyroid substance occurs as a result of inhibition of thyrotropin, largely because thyrotropic hormone is considered to be the chief regulator of thyroid activity. That the thyroid gland which is suppressed by exogenous thyroid hormone responds readily to stimulation by thyrotropin (6) is a convincing argument that endogenous thyrotropin activity is reduced by thyroxine. The ability of thyroid substance to effect a reduction in thyrotropin is suggested by experimental evidence in other species. For example, labelled thyroxine becomes concentrated in the pituitary gland in a few species (17), but not in the thyroid gland (18). Administration of thyroid hormone to rats decreases markedly the thyrotropin content of their pituitary glands (19). Lastly, diminution in the activity of thyrotropic hormone results from its incubation with thyroid tissue (20).

Although these ancillary observations suggest that thyroxine may inhibit the activity of thyrotropin, this action has not been demonstrated in the human subject by direct means, because of a limitation in methods for measuring endogenous levels of thyrotropin. Perlmutter and his associates (6) employed an indirect method of investigation by creating a state of excess thyrotropic activity in normal individuals. Administration of desiccated thyroid was shown to interfere slightly with total thyroidal collection of radioiodine during maximal stimulation of the thyroid gland. This led to the interpretation that exogenous thyroid hormone in the normal human subject exerted a major inhibitory effect on the activity of

thyrotropin, but a minor depressant action directly on the thyroid gland was also suggested.

An additional, equally indirect, approach to this problem lies in the consideration of thyroidal uptake in the presence of the reduced secretion of thyrotropin following hypophysectomy. In rats, a gradual decrease in thyroidal radioiodine uptake has been observed during the first week after hypophysectomy. A stable low uptake is attained by the fifth to seventh day. The decrement has been found to be fairly consistent, with reduction to about 60 per cent of the normal level by the first postoperative day, and to the range of 7 to 30 per cent by the fifth day (11, 12). That this results from lack of thyrotropin seems likely from the observations that thyroidal uptake rose to normal levels following administration of exogenous thyrotropic hormone. Of further interest is the fact that various degrees of radioiodine accumulation could be induced in a fractional manner with graded doses of thyrotropin (11). The limited data in Table 4 suggest that the reduction in thyroidal radioiodine collection following hypophysectomy in man is similar to that in experimental animals. The 2 hypophysectomized patients exhibited a decrease in thyroidal radioiodine accumulation to the hypothyroid range, on the fifth and sixth postoperative days, respectively. This rate of uptake was found to represent a value of 25 per cent of the preoperative rate in the 1 patient studied prior to hypophysectomy.

The data demonstrate that the depression of thyroidal radioiodine accumulation which occurs following abrupt administration of thyroxine to euthyroid subjects commences and reaches a maximum as rapidly as the decline which follows hypophysectomy. The absence of total suppression of uptake following administration of thyroxine is an additional point of similarity which, though little understood, is a well-documented occurrence following hypophysectomy (11, 12). These comparisons strongly suggest that a common mechanism controls the gradual diminution in thyroidal activity in both situations—namely an abrupt cessation of output of thyrotropin. Stanley and Astwood (4) demonstrated that the effect of a single dose of exogenous thyrotropin on the thyroidal collection of radioiodine in man persists for three to five days, with a gradual decline in intensity. It appears likely, then, that the gradual reduction in uptake during the several days following thyroxine administration or hypophysectomy results from utilization of thyrotropic hormone which had been released to the circulation prior to the termination of output.

Results of the present study indicate that thyroxine lacks significant thyroidal-depressing action in hypophysectomized individuals. The fact that administered thyroxine exerted no significant reduction in uptake in one patient studied, argues against a direct depressant action on the thy-

roid gland. Were the latter true, it would be reasonable to expect thyroxine to induce further and marked depression of uptake, in the virtual absence of circulating thyrotropin. In the other hypophysectomized subject, a fixed dose of thyrotropic hormone retained its full effectiveness in elevating thyroidal uptake after thyroxine was administered. Since each injection of thyrotropin resulted in a return of thyroidal accumulation toward the preoperative level but no higher, the dose employed would appear to represent the amount normally acting on the thyroid gland in this subject. The failure of thyroxine to interfere with this degree of thyroidal stimulation suggests that thyroid hormone possesses no inhibitory action on endogenous amounts of circulating thyrotropin in man.

The demonstrations that thyroxine is not a thyroidal depressant in the absence of thyrotropin and is unable to inhibit the stimulatory action of administered thyrotropin (in presumably endogenous quantity) strengthen the concept that exogenous thyroid hormone interferes with the output of thyrotropin. The wide variation in degrees of thyroidal depression, ranging from marked to none, observed in euthyroid subjects given thyroxine in this study may be considered to result from less than total inhibition of the release of thyrotropin by the quantity of thyroxine employed. Favoring this is the evidence previously cited, that thyrotropin administered in increasing doses to hypophysectomized rats induced a graded augmentation in thyroidal radioiodine collection (11).

Inasmuch as the quantity of thyroxine employed in the present study was considerably greater than physiologic, it is of interest that several euthyroid subjects exhibited slight or no decline in thyroidal radioiodine accumulation. Since their levels of thyroidal uptake prior to administration of thyroxine showed no constant difference as compared to those of other subjects, the effective quantity of circulating thyrotropin was presumably similar in all. The possibility arises that, at least in some individuals, thyroxine inhibition of thyrotropic hormone is a distinct phenomenon unrelated to the actual level at which thyrotropin output is normally set. This premise casts some doubt on the hypothesis that the quantity of thyroid hormone necessary to suppress thyroidal radioiodine accumulation is indicative of the normal endogenous output.

It seems permissible to suggest that the critical amount of exogenous thyroid hormone required to inhibit the release of thyrotropin varies widely in normal individuals. Whether it is comparable to the amount produced endogenously cannot be answered at present. If the endogenous output of thyroid hormone is considered equivalent to that required to maintain euthyroidism in athyreotic individuals (14), then it is clear that marked thyroidal depression is frequently effected in euthyroid subjects by administration of lesser quantities, and that occasionally inhibition is not

induced when greater amounts are given (5). Such a quantitative separation of the actions of thyroxine would serve as an explanation for the observation that when a fixed dose of 3 grains of desiccated thyroid is administered daily to euthyroid subjects, the serum protein-bound iodine level increases in some individuals but does not change in others (15). It seems tenable that in the former group suppression of thyrotropin is incomplete and the rise in serum protein-bound iodine level results from continuation of output of some endogenous hormone; whereas in the latter, inhibition of thyrotropin release is more marked.

Whether different preparations of thyroid hormone have similar potencies in inducing thyroidal suppression is not yet clarified. Starr and Liebhold-Schueck (7) showed that oral administration of sodium-*l*-thyroxine resulted in slightly greater depression than did sodium-*d*-thyroxine at equal dosage levels, but the differences were not great. In the amounts administered in this study, the effects of both the levo-rotatory and racemic preparations were similar, but no attempt was made to ascertain the minimally effective dosage levels.

It is probable that the transient inhibition of uptake occurring inconsistently within half an hour to two and three-quarter hours after injection of thyroxine bears no relation to the later marked depression. Since this early effect is abrupt in onset and termination, is short-lived, and does not alter the rate of accumulation permanently, it seems likely that the inhibitory agent is inorganic iodide liberated in small amounts from the thyroxine. The attempts to investigate this possibility did not exclude the possibility of an effect of small quantities of iodide on thyroidal uptake.

SUMMARY

Administration of a single dose of *dl*-thyroxine or *l*-thyroxine by the intravenous route to euthyroid adult subjects resulted in a depressive effect on thyroidal accumulation of radioiodine. In most subjects, this effect appeared within one day and became most marked at seven days after injection. Diminished thyroidal uptake persisted for one or more weeks. The elevation of serum protein-bound iodine to levels above the normal range following administration of thyroxine largely disappeared within one week.

Similarity in pattern was found between the depression of thyroidal radioiodine collection following thyroxine administration in euthyroid subjects and the decline in uptake which has been observed after experimental hypophysectomy in rats. In one hypophysectomized patient studied immediately before and six days after injection of thyroxine, no significant alteration in thyroidal radioiodine uptake was observed. In a second similar patient studied immediately before and six days after injection of thy-

roxine, no alteration was demonstrated in the response to thyrotropin injected in an amount sufficient to restore thyroidal radioiodine accumulation to the normal preoperative rate.

These observations are considered to support the concept that exogenous thyroid hormone administered to euthyroid subjects induces abrupt cessation of the output of thyrotropin. No evidence was obtained to indicate that thyroxine exerts an inhibitory effect on circulating thyrotropin or a direct depressant action on the thyroid gland.

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ALDOSTERONE EXCRETION IN HEALTHY PERSONS*

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IT IS now well established that aldosterone is excreted in the urine in man. This hormone was isolated from the adrenal cortex by Simpson *et al.* (1) in 1953. In the following paper we wish to report the daily variations encountered in healthy subjects and also some studies that have been carried out on the diurnal variation in the excretion of aldosterone. The urine was collected from healthy medical students and technicians, aged 22–33 years.

ASSAY OF ALDOSTERONE

Aldosterone was determined by means of a modification of the Singer-Venning (2) procedure which is dependent upon the excretion of sodium by adrenalectomized rats. Male hooded rats weighing between 165 to 180 grams are used for the assay. The animals are adapted for twenty-four hours to the conditions of a room kept at constant temperature (78° F.) and humidity (45 per cent) and are maintained on Purina Fox chow and tap water. At the end of this period the rats are bilaterally adrenalectomized by the dorsal route under light ether-anesthesia. The drinking fluid is changed to a solution of 1 per cent saline and 5 per cent glucose on the first postoperative day, and to 5 per cent glucose only on the second postoperative day. On the third postoperative day food is removed one hour prior to the assay. The animals are divided into groups of 10 rats each and under light anesthesia are given subcutaneous injections of the test substance or the solvent vehicle alone (0.1 ml. of aldehyde-free ethanol) and 1 ml. of an aqueous solution containing 3.5 mg. of sodium chloride. The urethra is ligated with a heavy thread and the rats are replaced in their cages and given free access to water during the remainder of the test. Exactly four hours after the injection they receive a lethal dose of Nembutal. The bladder is exposed and the urine is removed with a fine needle and syringe. Bladder and syringe rinses are added to the urine. The sodium content of the urine is determined by flame photometry. The mean sodium excretion of the control rats is considered to be 100 per cent excretion and that of the test animals is expressed as a percentage of the control value. The results are referred to the standard curve obtained with crystalline aldosterone. The sensitivity of the rats is checked by means of a standard dose of desoxycorticosterone acetate; the results are referred to the standard curve obtained with this substance and converted into aldosterone units. Aldosterone is 25 times more active than desoxycorticosterone acetate in this bioassay.

Preparation of urinary extracts. Urine is collected without preservative. A ten-hour

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aliquot of a 24-hour collection is used for the bioassay. The free fraction is first extracted from the urine with chloroform at pH 6. In the present study various methods of hydrolyzing the conjugate have been used. For acid hydrolysis the remaining urine is acidified to pH 1.5 and allowed to stand at room temperature for twenty-four hours. It is then re-extracted with chloroform. For enzyme hydrolysis, the urine is adjusted to pH 4.8, incubated for twenty-four hours with 200 units per ml. of β-glucuronidase,[1] and then extracted with chloroform. The free and hydrolyzed fractions are combined. The chloroform extract is washed twice with small amounts of N/10 alkali and water. The washings are back-extracted with chloroform, and the chloroform is evaporated off under reduced pressure.

The residue obtained is applied on a paper strip 5 cm. wide and run in the Bush B-5 system (3) (benzene-methanol-water) for seven hours at room temperature. Reference compounds hydrocortisone and cortisone are run on a pilot strip. The strips are cut so as to include material running from the end of the hydrocortisone band to the middle of the cortisone band. This paper strip is eluted with methanol. The methanol is evaporated to dryness and the residue is dissolved in a small quantity of absolute alcohol and sealed in a glass ampoule under light vacuum. An amount of extract equivalent to a forty-minute sample of urine is injected into each animal.

RESULTS

Normal range

Fifty estimations of aldosterone excretion were made on 12 healthy adult men and 12 estimations were made on 5 adult women. The values are shown in Figure 1. Twelve of these subjects carried out their usual activities during the collection of urine; 3 were hospitalized for certain investigations. In the males the average excretion was 3.2 μg. of aldosterone per twenty-four hours, the range being from 0.8 to 6.8 μg. In 2 subjects, values of 9.0 and 9.5 μg. were found on single days only. Since these high values were found on days prior to the administration of certain hormones for investigative purposes, the possibility that anxiety might influence the excretion has been considered. In the group of women, the average excretion of aldosterone was 3.8 μg. per twenty-four hours, the range being 1.7 to 5.5 μg.

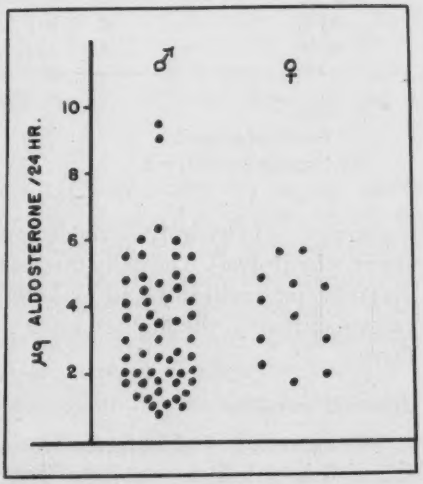

FIG. 1. Excretion of aldosterone in healthy adult males and females.

[1] We are greatly indebted to Warner Chilcott Laboratories for the liver β-glucuronidase (Ketodase) used in these experiments.

Daily variation

Eleven healthy subjects were followed over periods of three to eight days. The range of variation in aldosterone excretion is listed in Table 1. Most of the determinations were made on the free and acid-hydrolyzed fractions. In 2 cases the free and enzyme-hydrolyzed fractions were used for the bioassay, and in 1 case all three fractions were assayed. In our

TABLE 1. DAILY VARIATION IN URINARY EXCRETION OF ALDOSTERONE

Subjects	No. days	Fraction	Aldosterone (range) (μg./24 hrs.)
Males			
L.B.	6	F & A*	1.0–3.2
P.M.	7	F & A	1.0–4.6
O.R.	6	F & A	1.0–4.9
J.B.	7	F & A	0.8–9.3
C.G.	3	F & A	2.6–4.1
J.S.	8	F & A	0.9–4.5
P.P.	3	F & E	1.7–6.0
J.P.	5	F, E & A	2.3–9.5
Females			
I.D.	3	F & A	3.1–4.9
M.L.	3	F & A	2.0–5.6
E.W.	3	F & E	1.7–4.8

* F = Free.
A = Acid-hydrolyzed.
E = Enzyme-hydrolyzed.

experience, acid hydrolysis yields greater amounts of aldosterone than does enzyme hydrolysis. The daily fluctuations are somewhat greater than those observed for corticoids and 17-ketosteroids. With the exception of the 2 cases mentioned, the highest value does not exceed 7 μg. per twenty-four hours.

Diurnal variation

The excretion of aldosterone during the day and the night periods was followed in 9 healthy subjects. The values are shown in Table 2. In 6 of the cases a higher excretion of aldosterone was observed during the day; in the 3 others, trends were inconsistent. In the cases in which 17-hydroxy-corticosteroids were measured, there was a consistently higher output during the day than during the night.

TABLE 2. DIURNAL URINARY EXCRETION OF CORTICOSTEROIDS

Normal subjects	Aldosterone		17-OH-Corticosteroids	
	Day (μg./12 hrs.)	Night (μg./12 hrs.)	Day (mg./12 hrs.)	Night (mg./12 hrs.)
Males				
1. G.	1.9	0.8	—	—
	1.9	4.0	4.2	1.2
	0.8	3.3	3.6	1.8
2. PA.	4.5	1.4	3.2	2.4
	4.0	2.0	3.0	2.4
3. H.	4.5	2.3	2.6	1.0
4. P.	0.7	1.0	—	—
	1.6	2.3	—	—
	3.8	2.1	—	—
5. A.	1.1	0.8	3.0	2.0
	2.7	1.6	2.8	1.8
6. S.	1.2	5.2	7.6	4.2
Females				
1. L.	2.1	1.5	—	—
2. D.	2.0	1.0	2.0	0.6
	3.0	1.7	2.4	0.4
	3.7	1.2	1.6	0.6
3. W.	2.2	2.0	—	—
	1.6	1.4	—	—
	1.1	1.0	—	—

Effect of stress or anxiety

In certain individuals, who were being followed from day to day, unexpectedly high excretions of aldosterone were observed on several occasions. These high values could be correlated with anxiety or some emotional disturbance. In order to determine whether this might be the explanation, urine was collected from 3 students, during and after an examination. All 3 showed higher excretions of aldosterone on the day of stress. These findings are shown in Table 3. In one subject an increase in aldosterone was observed following a period of acute anxiety, and in another following an emotional disturbance.

DISCUSSION

Because of the uncertainty of the nature of the material assayed for sodium-retaining activity, in published reports the excretion of aldosterone has been expressed in a variety of ways, such as a percentage of the control-group values either in terms of sodium excreted or the Na-K ratio; as μg.

TABLE 3. EFFECT OF STRESS UPON ALDOSTERONE EXCRETION

Day	Case 1	Case 2	Case 3	Case 4	Case 5
1	—	—	—	—	3.0
2	—	—	2.6	2.0	2.6
3 *Stress*	6.9*	8.7*	6.0*	20.0†	6.4‡
4	4.9	6.5	4.1	3.2	1.8
5	3.6	2.5	—	5.6	—
6	3.3	1.3	—	4.8	—

* Examination.
† Acute anxiety.
‡ Emotional disturbance.

of desoxycorticosterone per day; and in a few instances as aldosterone. It is therefore difficult to compare values obtained in different laboratories. In the earlier work, assays were carried out on crude neutral extracts without purification of the extract by chromatographic procedures, and in many instances inadequate methods of hydrolysis were employed. Under these conditions little or no sodium-retaining activity could be demonstrated in normal urine.

Luetscher *et al.* (5) demonstrated that the sodium-retaining substance present in the urine of patients with nephrosis is aldosterone. The fraction used for the assay of sodium-retaining activity in our studies contains material running with the same rate of flow as aldosterone, and includes some cortisone. This latter steroid, in amounts found in normal urine, has no effect on the sodium-retaining activity of aldosterone in our assay, although in larger amounts it causes sodium excretion. So far, aldosterone is the only known corticosteroid in this fraction which has potent sodium-retaining activity. The possibility that small amounts of unidentified compounds which affect sodium metabolism may also be present cannot be excluded. There is some indication that in certain urinary extracts, even after purification by chromatography, there may be substances which interfere with the assay of aldosterone.

Using the method of extraction and hydrolysis as described, our range for healthy adults is somewhat greater than that already reported. This is probably due to the fact that we have studied a greater number of individuals and have followed many over longer periods of time. Using acid hydrolysis for a period of twenty-four hours and purification of the extract in the toluene and propylene-glycol system, Axelrad *et al.* (6) found the normal output of aldosterone to be in the range of 2–4 μg. per twenty-four hours. Genest (7), using β-glucuronidase hydrolysis and purification by

two chromatographic systems (Zaffaroni and Bush C), failed to find significant amounts of aldosterone (<1.4 μg.) in normal urine.

The studies of Luetscher *et al.* (8) indicate that aldosterone excretion is a reflection of a normal mechanism for controlling sodium metabolism. In acute conditions, such as salt deprivation, increases in the output of this hormone can be demonstrated. Some of our subjects maintained on a relatively constant sodium intake showed considerable fluctuation in aldosterone excretion, and it is difficult to relate these variations to variations in sodium metabolism only. Although the role of the anterior pituitary in releasing aldosterone from the adrenal cortex appears to be a minor one, in several of the cases studied an increase in aldosterone excretion could be associated with a known stress. It is obvious that at the present time the number of cases studied is too few to allow any deductions to be drawn regarding the effect of stress on aldosterone excretion.

In an earlier study (9) carried out on 4 normal subjects no consistent trend could be observed in the diurnal excretion of aldosterone. We now have completed 19 studies on 9 subjects, and in 14 of these the day specimen showed a higher content of aldosterone than the night specimen. This suggests that in the majority of cases there is a diurnal rhythm in the excretion of aldosterone, similar to that observed for the corticosteroids and the 17-ketosteroids.

CONCLUSIONS

The excretion of aldosterone in the urine of healthy adult subjects has been determined by means of a bioassay based on the excretion of sodium in adrenalectomized rats.

The mean of 50 determinations of aldosterone excretion in 12 normal men was 3.2 ± 1.6 μg., and of 12 determinations in 5 normal women, 3.8 ± 1.4 μg. per twenty-four hours. There was considerable variation from day to day in the same individual. In 6 of 9 subjects there was a diurnal rhythm. In 5, an increased excretion of aldosterone was associated with a stressful situation.

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AN IMPROVED PROCEDURE FOR THE DETERMINATION OF HYDROCORTISONE IN HUMAN PLASMA

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IN THE procedure of Silber and Porter (1) for the determination of 17,21-dihydroxy-20-ketosteroids in plasma, the addition of standard hydrocortisone to the samples was recommended, although not essential. It was also pointed out that greater sensitivity could be obtained if a smaller volume of reagent were used and if the solvent extract were reduced in volume prior to addition of reagent. Automatic correction for the incomplete extraction of steroid from plasma was made by employing standards in chloroform-washed plasma.

From the analyst's point of view, the addition of standard to the samples and the use of plasma standards are undesirable features. Therefore, another procedure has been devised, eliminating these two features and increasing the sensitivity of the method. Steps have been introduced to eliminate carbon tetrachloride and petroleum ether-soluble substances (fat, certain drugs, and other steroids) from the sample prior to extraction of hydrocortisone, and provision has been made for the elimination of acetone, which may be present in the plasma of diabetics.

MATERIALS

Methylene chloride (reagent, Merck).

Carbon tetrachloride (reagent, Merck).

Petroleum ether (reagent, Merck, b.p. 35°–75° C.).

0.1 N sodium hydroxide.

Phenylhydrazine hydrochloride, recrystallized from ethanol and dried over calcium chloride.

Sulfuric acid (dilute)—190 ml. of water plus 310 ml. of concentrated acid (reagent, Merck).

Blank reagent—100 ml. of dilute sulfuric acid mixed with 50 ml. of absolute ethanol.

Phenylhydrazine-sulfuric acid reagent with ethanol—65 mg. of phenylhydrazine hydrochloride dissolved in 150 ml. of blank reagent.

Hydrocortisone standard (5 μg. per ml.) diluted before use to 0.5 μg.-3.0 μg. per ml.

Centrifuge tubes, 45–50 ml. capacity, to which plastic caps can be fitted.

Test tubes, about 180 mm. long with 15 mm. inside diameter, to which plastic caps can be fitted, calibrated at 10 ml. (blood sugar tubes can be used).

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Small test tubes, about 100 mm. long with 10 mm. inside diameter (or 12–15 ml. centrifuge tubes).

Plastic caps, No. 590, Lumelite Corp., Pawling, N. Y.

Blunt-tip needles, about 7 inches long, 16 or 18 gauge.

Microcuvettes for the Beckman model DU spectrophotometer—silica cells, with 3×10×38 mm. inside dimensions, and diaphragm attachment (smaller cuvettes with a 10-mm. light path can be used).

PROCEDURE

1. A 12-ml. or larger sample[1] of plasma is washed with 2½ volumes of carbon tetrachloride by shaking for fifteen seconds in a centrifuge tube equipped with a plastic cap. After centrifugation, the solvent layer is discarded and 2½ volumes of petroleum ether are added. Again, after the tube is shaken for fifteen seconds and centrifuged, the solvent layer is discarded.
2. A 10-ml. sample of the washed plasma is now extracted in a second centrifuge tube by shaking for twenty to thirty seconds with 25 ml. of methylene chloride. After centrifuging once, the tube and contents are immersed in a dry ice-solvent mixture[2] until the aqueous phase is frozen, and centrifugation is repeated.
3. The solvent layer is transferred, by means of a blunt-tip needle and a 10-ml. syringe, to a 180-mm. test tube calibrated at 10 ml. Methylene chloride (10 ml.) is added to the tube containing the now thawed plasma.
4. The first extract (22–24 ml. of solvent) is evaporated to a small volume in a stream of air in the 180-mm. tube immersed in a 25–30° C. water bath.
5. The second extraction (with 10 ml. of solvent) is carried out like the first, and the solvent extract is transferred with the same syringe to the calibrated tube containing the residue from the first extraction. The volume is carefully adjusted to 10 ml. by either evaporating in air or by adding a small amount of methylene chloride, as needed.[3]
6. One ml. of 0.1 N NaOH is added and after the tube is capped it is shaken for fifteen seconds and centrifuged. The alkaline wash is discarded and a 4-ml. aliquot of the extract is placed in each of two small test tubes. To one is added 0.5 ml. of phenylhydrazine reagent and to the other, 0.5 ml. of blank reagent. The tubes are then shaken vigorously for fifteen seconds with the thumb over the top of the tube. A rubber finger-cot (rinsed carefully with water and solvents) is worn to protect the thumb and prevent contamination of the sample.
7. After centrifugation, the solvent is removed (and discarded) with a blunt needle connected to an aspirator.
8. After standing overnight, optical densities of the sample and its blank are measured at 410 mμ. Aqueous standards are carried through the same procedure. Cuvettes are rinsed with ethanol and allowed to drain thoroughly between samples.

Notes:

A. All glassware, needles, and caps are washed with water, alcohol, and methylene chloride before use.

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[1] If a 5-ml. sample is to be analyzed, about 7 ml. of plasma can be used here.

[2] Suggested by Mr. Alfonso Orr of the Creedmoor Institute for Psychobiologic Studies, Queens Village 27, N. Y., to break the emulsion.

[3] If acetone is present, the total extract is taken to dryness and 10 ml. of fresh methylene chloride added before introducing the 0.1 N NaOH.

B. When centrifuging the 180-mm. test tubes the speed of the centrifuge must be increased slowly until the tubes reach a horizontal position.

SENSITIVITY

Both 10-ml. and 5-ml. samples of a pooled normal plasma were analyzed in triplicate on two occasions. Aqueous standards were also analyzed in triplicate, 0.5 μg., 1.0 μg., and 2.0 μg. each.

The amounts of hydrocortisone found were 1.90 ± 0.03 (S.E.) μg. for the six 10-ml. samples and 1.05 ± 0.024 (S.E.) μg. for the six 5-ml. samples. The slightly higher concentration found after analysis of the 5-ml. samples may have been due, at least partly, to better recovery of the steroid from the smaller sample.

RECOVERY

Ten-ml. portions of another sample of plasma were analyzed in triplicate, with and without addition of 0.5 μg. or 1.0 μg. of hydrocortisone. The plasma was found to contain 1.61 μg. per 10 ml.; 2.07 μg. after the addition of 0.5 μg. of standard; and 2.52 μg. after the addition of 1.0 μg. of standard. Thus, the recovery of added steroid was 0.46 μg. of 0.5 μg. (92 per cent), and 0.91 μg. of 1.0 μg. (91 per cent). Therefore, the corrected value for this sample of plasma would be 1.76 μg. in 10 ml.

CALCULATIONS

The following are typical data:

Optical density at 410 mμ

Plasma	*Sample*	*Blank*	*Difference*
10 ml.	0.076	0.014	0.062
	0.074	0.013	0.061
	0.092	0.029	0.063
Standards			
2 μg.	0.075	0	0.075
	0.074	0	0.074
	0.079	0	0.079
			Av. 0.076

$$\frac{2}{0.076} \times 0.062 = 1.63 \text{ μg./10 ml.}$$

$$\times 0.061 = 1.61 \text{ μg./10 ml.}$$

$$\times 0.063 = 1.66 \text{ μg./10 ml.}$$

DISCUSSION

This procedure is more tedious than the one previously described (1) but appears to yield more satisfactory results. It has even been possible to

apply it to 2-ml. samples of normal plasma (20 μg. per 100 ml.) with a fair degree of accuracy.

It is desirable to make additional readings at 380 and 440 mμ. If substances such as acetone, paraldehyde, or bile (2, 3) are present, it will then be obvious that the determination is invalid and steps should be taken to eliminate the interfering substance. The procedure in its present form cannot be applied to plasma from jaundiced patients.

The analyst can vary the procedure in a number of ways, if he wishes. A single extraction can be made, followed by evaporation to a smaller volume prior to addition of reagent. The volume of reagent can then be decreased to any volume that can be handled quantitatively and read in appropriate cuvettes. For practical purposes, however, it is felt that the 0.5-ml. volume of reagent and use of the cells with a light path 10 mm. long and 3 mm. wide are most satisfactory. It is also possible to omit either or both solvent washings prior to steroid extraction, if the fat content of the sample is low and no solvent-soluble medication has been administered.

SUMMARY

A modification of the Silber and Porter (1) procedure for the determination of hydrocortisone in plasma has been described. This modification has greater sensitivity and avoids the addition of standard to the samples, as well as the use of standards in plasma. Provision has been made for the analysis of plasma from patients with diabetes mellitus.

Pooled human plasma assayed 19 μg. per 100 ml. when 10-ml. portions were used, with a standard error of 0.3 μg. Five-ml. samples of the same plasma assayed 21.0 μg. per 100 ml., with a standard error of 0.48 μg.

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HISTOLOGIC CRITERIA FOR DETECTION OF PROLACTIN: LACK OF PROLACTIN IN BLOOD AND URINE OF HUMAN SUBJECTS

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INTRODUCTION

THERE are two widely used methods for bioassay of prolactin which utilize the response of the pigeon's cropsac. In the systemic method (1, 2) prolactin is injected subcutaneously or intramuscularly into the pectoral region of the pigeon. In the local method (3–6) prolactin is injected intradermally over the cropsac. Both of these methods have provided useful and reliable data when applied to studies of anterior pituitary preparations. On the other hand, assays for prolactin in human urine and serum, chiefly by the local assay method, have yielded conflicting results.

In 1941 Meites and Turner (7), using the local assay method, reported that postpartum women excreted 4–12 I.U. of prolactin per twenty-four hours and that pregnant patients excreted about 10 per cent of this quantity. Their review cited other authors who reported the presence of similar quantities of prolactin in human urine and serum. In 1943, Lahr, Bates and Riddle (8) questioned the results of local assays of urine and serum when they demonstrated that focal epithelial proliferation, the endpoint of most local assays, can also be induced by the local intradermal injection of many nonspecific irritants.

To circumvent the problem of nonspecific local cropsac stimulation, Segaloff and associates (9–11) described a systemic bioassay in which urine extracts were injected intravenously into pigeons that were also receiving near-threshold subcutaneous booster doses of purified prolactin. Using this procedure, they reported that the daily urinary excretion of prolactin by normal men and women was 0–305 I.U. and 52–151 I.U., respectively. Women in the late stages of pregnancy, postpartum women and women with persistent lactation usually excreted over 200 I.U. per twenty-four hours. The high values obtained by this assay method are unique, in that they are ten times greater than the values reported by other authors (7).

The purpose of this study was to re-examine the assay of prolactin in

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urine and serum by the local method. Histologic and tinctorial criteria characteristic of prolactin stimulation were first established. When these criteria were applied to local cropsac assays, prolactin activity was demonstrated in the human anterior pituitary, but not in human serum or urine.

MATERIAL AND METHODS

Immature white Carneau pigeons, 7–10 weeks old, were used in all the tests. The substances tested were administered in distilled water or in 0.8 per cent sodium bicarbonate solution by daily intracutaneous injections (0.1–0.5 ml. volume) for four days.

The dissected cropsacs were graded by gross inspection (0 to 4 plus), then stretched over circular cardboard frames (diameter, 4 cm.) and immediately fixed for twenty-four to forty-eight hours in 10 per cent neutral formalin. Paraffin sections were prepared from a representative portion of the cropsac and stained with azure-eosin (pH 4.5; 60 min.) (12).

A preparation of sheep prolactin having a potency of 20–25 I.U. per mg. was used as the standard. Human anterior pituitary powder represented a combination of the lyophilized powder from ten glands. The glands were removed from male and female adult patients within eight hours following death. The patients presented no clinical evidence of endocrinopathy.

Samples of urine and serum were obtained from adult patients—one postpartum, another pregnant, and others who might have been expected to excrete prolactin in the urine (10, 13). Serum solids were recovered by lyophilization. Urine was concentrated to about 0.1 volume by lyophilization, or in a flash evaporator. The concentrate was then dialyzed and the nondialyzable solids recovered by lyophilization. In one experiment 100 I.U. (5 mg.) of prolactin was added to the urine before processing.

The nondialyzable urinary solids of Patient M.R.B. (Table 1) were fractionated into a pH 5.5 soluble fraction, I-A (1.1 Gm.); the pH 5.5 insoluble fraction was divided into a pH 9.5 soluble fraction, I-B (600 mg.), and a pH 9.5 insoluble fraction, I-C (700 mg.). Any prolactin, if present, was expected to be in I-B. A portion of the urinary solids of Patient J.N. was fractionated with methanol into a neutral-methanol soluble fraction, II-B (11 mg.), and an acid-methanol soluble fraction to which 5 volumes of acetone were added, causing a precipitate, II-D (9 mg.). The acid-acetone soluble fraction was then neutralized, yielding a precipitate, II-E (16 mg.). The fraction insoluble after extraction with both neutral and acid methanol was II-A (44 mg.). Any prolactin was expected to be in II-D or II-E.

The diagnoses, weights of solids recovered, weights of solids assayed, and number of tests performed, are recorded in Table 1.

RESULTS

I. *Responses of the Pigeon Cropsac*

Effects of purified prolactin

The cropsac can easily be separated from the underlying tissues by blunt dissection through a cleavage plane passing through the loose connective tissue surrounding the cropsac. This dissection preserves the epithelium, the tunica propria, and the muscularis. The normal mucosa (Fig. 1) is lined by a few (two to five) layers of thin stratified squamous epithelium.

TABLE 1. EFFECTS OF EXTRACTS OF URINE AND SERUM ON CROPSAC EPITHELIUM

Diagnosis	Patient and sex	Material injected	Solids		No. of pigeons	Max. gross thickening of epith.	Diffuse basophilia
			Recovered (mg./24 hrs.)	Amount assayed (mg.)			
Post partum, 24–72 hrs.	M.R.B. (f)	NDUS*	2400	—	—	+	—
		UF†-IA	1100	2–10	2	+++	0
		UF-IB	600	2–10	2	++++	0
		UF-IC	700	10	1	++	0
		Serum	—	1–85	7	+++	0
Pregnancy, 38th week	B. (f)	NDUS	1200	2–10	4	++++	0
Galactorrhea and amenorrhea	N.G.	NDUS	800	1–20	14	++++	0
		Serum	—	7–30	5	++	0
Galactorrhea and amenorrhea	S. (f)	NDUS	750	1–5	4	++++	0
Gynecomastia	J.N. (m)	NDUS	1500	0.2–20	8	++++	0
		UF-IIA	264	0.2–1	2	++++	0
		UF-IIB	66	0.2–1	2	0	0
		UF-IID	54	0.2–1	2	++	0
		UF-IIE	96	0.2–1	2	0	0
Gout; purine-free diet	R. (m)	NDUS	2400	2–10	2	++	0
Thyrotoxicosis	C. (f)	Serum	—	7–28	2	++++	0
Pooled human plasma		Plasma	—	3–60	4	++	0

* Nondialyzable urinary solids.
† Urinary fraction.

These cells show virtually no mitotic activity. Their cytoplasm is not basophilic.

The effects of various doses of purified prolactin administered intradermally over the cropsac in four equal daily doses of 0.5 ml. are recorded in Table 2. Some hypertrophy of squamous cells was observed in pigeons

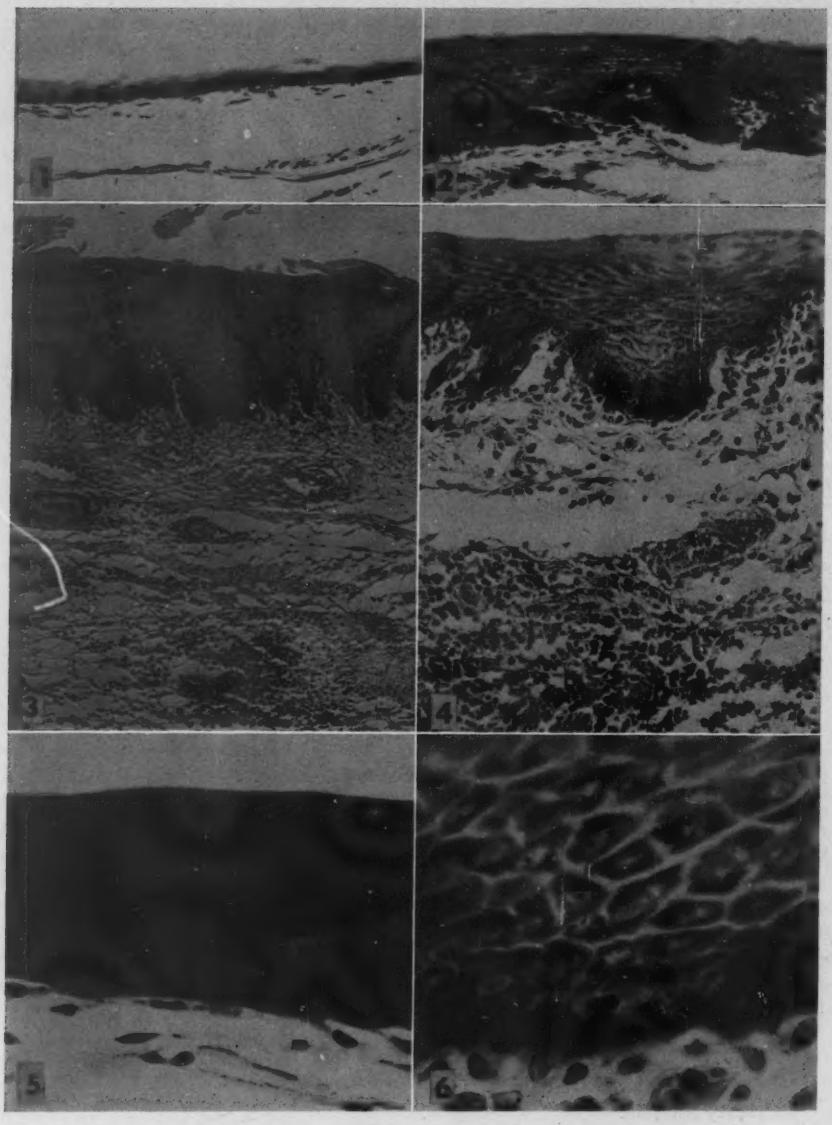

FIG. 1. Normal pigeon cropsac (azure-eosin (A-E); ×215). Epithelium consists of 2–4 layers of thin squamous cells.

FIG. 2. Effects of total dose of 2 μg. of purified prolactin (A-E; ×215). Epithelium consists of 4–8 layers of cells. Intense diffuse cytoplasmic basophilia obliterates cellular details.

FIG. 3. Effects of 0.5 ml. of 30 per cent ethanol (A-E; ×105). Epithelium consists of

receiving a total dose of less than 0.5 μg. of purified prolactin. Doses of at least 0.5 μg. of prolactin not only caused varying degrees of hyperplastic thickening of the epithelium accompanied by increased mitotic activity, but also invariably caused distinct diffuse cytoplasmic basophilia. Under the conditions of the staining procedure, this basophilia was so dense that individual nuclei and cell membranes were difficult to identify (Figs. 2

TABLE 2. EFFECTS OF PURIFIED PROLACTIN ON CROPSAC EPITHELIUM

Total dose of prolactin		No. of pigeons	Gross thickness	Focal histologic responses	
Micrograms	I.U.			Thickness (no. cells)	Diffuse basophilia
0.00	0.000	2	0	2–5	0
0.03	0.001	2	0	2–5	0
0.10	0.003	2	0	2–5	0
0.20	0.005	1	0	2–5	±
0.25	0.006	2	±	2–5	±
0.32	0.008	2	+	2–8	±
0.50	0.013	4	+	4–8	+
1.00	0.025	6	++	4–10	+
2.00	0.050	5	+++	5–10	+
4.00	0.100	3	++++	5–15	+
10.00	0.250	1	++++	10–20	+

and 5). The basophilic material of the cytoplasm could be removed from tissue sections by incubation with ribonuclease or extraction by mineral acids prior to staining. These procedures remove ribonucleic acids from tissue sections (12). Purified prolactin caused the formation of large neutral lipid droplets within epithelial cells. Little or no inflammation of the tunica propria was observed.

5–20 layers of cells and cytoplasmic basophilia is confined to basal zone. There is marked edema and inflammation of underlying tissues.

FIG. 4. Effects of total dose of 6 mg. of nondialyzable urinary solids of Patient B., in 38th week of pregnancy (A-E; ×205). Epithelium consists of 5–10 layers of cells and cytoplasmic basophilia is confined to basal zone. There is marked edema and inflammation of underlying tissues.

FIG. 5. Effects of total dose of 4 mg. of nondialyzable urinary solids of Patient B., plus 5 μg. of purified prolactin (A-E; ×1380). Intense diffuse cytoplasmic basophilia obliterates cellular detail.

FIG. 6. Effects of total dose of 4 mg. of nondialyzable urinary solids of Patient B., plus 2 μg. of prolactin (A-E; ×1380). There is some inhibition of prolactin effect by urinary solids.

Effects of nonspecific substances

The effects of a group of nonspecific irritants when injected locally, are listed in Table 3. Most of these substances caused varying degrees of gross and microscopic thickening of the epithelium accompanied by distinct edema and inflammation of the tunica propria (Fig. 3). In contrast to the effects of prolactin, the irritants did not cause diffuse cytoplasmic basophilia. The cytoplasmic basophilic material which was present was distributed about the nuclei of the cells in the basal half of the epithelium. Individual nuclei and cell membranes could easily be identified. Individual cells appeared to be separated by intercellular fluid and in some areas intercellular bridges were observed.

TABLE 3. EFFECTS OF INJURY ON CROPSAC EPITHELIUM

Agent	Total dose	Days	Gross thickness	Focal histologic responses	
				Thickness (no. cells)	Diffuse basophilia
Penetrating wound	—	4	++++	4–20	0
Ethanol, 30%	0.5 ml.	4	++++	4–20	0
Tannic acid	10 mg.	4	+++	2–10	0
Pseudomonas polysaccharide*	8 μg.	4	++	3–6	0
Mustard	5 mg.	2	++	3–6	0
0	0	0	0	2–4	0

* Piromen—Travenol Laboratories, Inc., Morton Grove, Illinois.

II. *Prolactin Assays*

Human pituitary tissue

Pooled human anterior pituitary powder was injected locally at total-dose levels ranging from 0.1 to 10 mg. All dosages caused epithelial proliferation accompanied by marked diffuse cytoplasmic basophilia. The proliferation caused by 0.1 mg. of human pituitary powder was about equal to that produced by 5 μg. of purified prolactin. Therefore, the potency of the pooled human anterior pituitary powder was approximately 1.0 I.U. per mg. of powder.

Human serum and urine

The results of all the assays by the local method on human serum and urine were essentially negative and are listed in Table 1. All extracts caused moderate to marked inflammation accompanied by distinct gross and microscopic epithelial thickening at higher dosages (Fig. 4). When present,

basophilic material was perinuclear (Fig. 6). Diffuse cytoplasmic basophilia was not observed in any assay. Thus, the responses of the cropsac in this series of tests were qualitatively identical with those induced by nonspecific irritants (Fig. 3). In no assay was there any histologic evidence of the presence of prolactin. In assays of urinary fractions of Patients M.R.B. and J.N., the material causing epithelial proliferation was not concentrated in the fractions in which prolactin might be expected to occur.

Recovery of prolactin from urine extracts

Several experiments were carried out using the local method of assay to determine the amount of prolactin activity which could be detected when the purified hormone was added to nondialyzable urinary solids and to fresh urine. It was found that the inflammatory reaction partially obliterated the typical histologic features of the cropsac response. Nevertheless, diffuse basophilia was detected when added prolactin comprised at least 0.2 per cent of the total solids; for example, the presence of 5 μg. of purified prolactin could be detected in 5 mg. of nondialyzable urinary solids. Moreover, upon addition of 100 I.U. of prolactin to fresh urine, the activity of prolactin could be discerned by the histologic tests after local cropsac injection of as little as 3 mg. of total nondialyzable solids.

Correlation of systemic with local assays

The following intramuscular injections were made—each into one pigeon over a four-day period: 1) 65 per cent of the 24-hour urinary extract from Patient S; 2) 90 per cent of the 24-hour urinary extract from Patient B; and 3) 4 ml. of serum from Patient M.R.B. In no case was there any thickening of the cropsac. Since 4 I.U. of prolactin will stimulate a positive response in 50 per cent of a group of pigeons, these systemic tests indicate that the 24-hour urine samples contained less than 5–10 I.U. and that the serum contained less than 1.0 I.U. per ml. When 100 I.U. of prolactin was added to fresh normal urine before concentration, the total prolactin activity was recovered quantitatively from the nondialyzable solids on the basis of intramuscular assay in 4 pigeons. One-fifth of the sample (equivalent to 20 I.U.) induced growth of cropsacs to a weight of 4.24 ± 0.44 Gm. Control cropsac weight was 1.2 Gm. This experiment with added prolactin demonstrates that with the preparative methods employed it is possible to recover pituitary prolactin from urine if it is present, and that injected urinary extracts do not interfere with the absorption of prolactin from the site of injection.

DISCUSSION

These data are consistent with the general experience of one of us (R.W.B.) over the last twenty years and illustrate the following facts: 1)

If assayed by the systemic method, urine or serum extracts have never been shown to cause any changes in the pigeon's cropsac epithelium when such preparations were injected without a booster dose of prolactin; 2) In assays of urinary fractions the material associated with marked proliferative changes, observable both grossly and histologically, is not concentrated in the fractions of urine in which prolactin would be expected. 3) In local cropsac assays, diffuse cytoplasmic basophilia cannot be induced by serum, whole-urine powder, or any fractions thereof. Thus, there seems to be no unequivocal biologic or biochemical evidence to establish the presence of measurable amounts of pituitary prolactin in human serum or urine.

It is probable that the substances of serum and urine which stimulate the cropsac locally are nonspecific irritants, possibly mucopolysaccharides. Although not prolactin, this unknown material may have some clinical significance, for it does seem to increase in such conditions as pregnancy, lactation and galactorrhea.

The positive results of systemic-type assays involving booster doses of prolactin are more difficult to reconcile with our data. The well established variations of the cropsac response with race of pigeon (14), season (15) and route of injection (16), along with the biologic variation of the individual pigeon, may account for a certain number of "false positive" results in booster-type assays. The possibility that human urine contains a compound which, when injected intravenously, augments the response of the cropsac to threshold doses of prolactin, cannot be entirely disregarded. If such a compound exists, however, these data indicate that it is not pituitary prolactin.

The diffuse cytoplasmic basophilia caused by prolactin is of some basic importance, for it indicates that prolactin alters the distribution of ribonucleic acids within the epithelium of the pigeon's cropsac. The dense basophilia probably can be regarded also as a crude indication of a concomitant increase in the concentration of ribonucleic acid within the epithelium of the cropsac. This interpretation is strengthened by the observations of Brown *et al.* (17), and McShan *et al.* (18). The former group showed that the concentrations of P^{31} and P^{32} in the pigeon's cropsac are increased within eight hours following the intramuscular injection of prolactin. The latter group reported that the daily intramuscular injection of prolactin for five days caused a marked progressive daily increase in the concentration of pentose nucleic acid of the pigeon's cropsac. Many investigators have correlated increases in cytoplasmic ribonucleic acid with active protein synthesis (19). Considering that a normal brooding 500-gram pigeon each day synthesizes up to 25 grams of crop milk containing 15 per cent protein and 15 per cent fat (20), this correlation between protein synthesis and basophilia in the prolactin-stimulated cropsac is expected.

SUMMARY AND CONCLUSIONS

Under the stimulus of either purified prolactin or human anterior pituitary powder the epithelial cells of the pigeon's cropsac not only increased in number, but also developed intense cytoplasmic basophilia. In contrast, human urine concentrates, serum, and nonspecific agents (such as ethanol, tannic acid, and pseudomonas polysaccharide) failed to stimulate the cropsac when injected intramuscularly. All caused epithelial thickening of the cropsac when injected intradermally over the crop, but never induced diffuse cytoplasmic basophilia. On the basis of these observations, it is concluded that the urine and serum of the patients studied contained little or no prolactin (<5 I.U. per twenty-four hours), although in some of the patients there was clinical evidence of endogenous prolactin stimulation.

The validity, specificity and sensitivity of the methods employed are illustrated by recovery experiments and by parallel assays of urine extracts and serum by the systemic method.

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STUDIES ON THE SALIVARY SECRETION OF IODIDE*

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SEVERAL reports have dealt with salivary concentration of I^{131} (1, 2, 3). The degree of this concentration is similar to that reported for the thyroid gland (4, 5, 6). An additional point of similarity is that the mechanism is inhibited in both the salivary gland and in the thyroid (7) by certain anions. The nature of this mechanism and the question of whether organically bound plasma iodine contributes to salivary iodine are topics of current interest.

The ratios of salivary I^{131} to plasma inorganic I^{131}, immediately and twenty-four hours after the administration of I^{131}, have been reported by others (1, 8). The present study extends these observations for periods up to ten days in 13 subjects, and includes data from 4 patients who were given I^{131}-labeled thyroxine.

MATERIALS AND METHODS

After a preliminary mouth rinse of tap water, the patient was asked to chew on a small piece of paraffin for two minutes. The paraffin and accumulated saliva were then discarded and all subsequent saliva was collected in a centrifuge tube until a total of 4 ml. had been obtained. The I^{131} activity of whole plasma and of saliva was measured in a calibrated well-scintillation (γ) counter. The precipitable I^{131} activity was determined by the method of Sheline and Clark (9), adapted for the well counter. The plasma inorganic I^{131} activity was obtained by difference.

The variability attributable to the method of collection and measurement of salivary activity was estimated by collecting 3 samples at thirty-minute intervals from the same patient. This was carried out on several patients until a total of 40 consecutive daily samplings was obtained. Analysis showed that the coefficient of variation averaged 15 per cent.

In Table 1 is a summary of the pertinent clinical information concerning the patients who cooperated in this study.

RESULTS

Table 2 shows the ratios of salivary I^{131} to plasma-nonprecipitable I^{131} (SI^{131}/PI^{131}) at twenty-four and forty-eight hours for patients with hypo-

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TABLE 1. SUMMARY OF CLINICAL AND LABORATORY INFORMATION

Patient	Sex and age	BMR (%)	PBI (μg./100 ml.)	Thyroidal uptake of I^{131} (% in 24 hrs.)	Urinary excretion of I^{131} (% in 24 hrs.)	Diagnosis and case summary
W.C.	M52	−16	0.7	0.18	19.1	Multiple myeloma; myxedema for 6 mos. due to admin. of I^{131}.
L.D.	F29	−18	2.33	—	79.7	Total thyroidectomy in 1951 for thyroid cancer; 350 mc I^{131} during interval since op.; some residual functioning tumor.
H.B.	F28	−19	3.50	—	52.8	Total thyroidectomy in 1952 for thyroid cancer; 30 mc I^{131} in 1952; thyroid medication discont'd 2½ wks. prior to study.
N.G.	F41	−27	2.80	— —	44.0 70.1	Radical thyroidectomy in 1955 for thyroid cancer.
J.W.	F64	+1	6.53	15.3	47.2	Recurrent nodular goiter; clinically euthyroid.
B.B.	F40	−15	5.37	2.8 1.3	70.9 66.7	Anxiety reaction; propylthiouracil discontin'd 4 days prior to test; clinically euthyroid.
C.M.	F62	−6	3.5	— —	43.1 36.4	Polycythemia rubra vera; arteriosclerotic heart disease; clinically euthyroid.
S.M.	F49	—	9.8	29.1 (56 hrs.)	37.7	Multiple myeloma; clinically euthyroid.
A.P.	F41	+27	15.6	—	44.0	Thyrotoxicosis; no previous treatment.
R.T.	M26	+9	6.8	(75 ml./min. thyroidal clearance)	36.3	Toxic nodular goiter; prior treatment included propylthiouracil and stable iodine; clinically thyrotoxic.
O.W.	M68	+28		56.7	7.6	Nodular toxic goiter; propylthiouracil discont'd 3 wks. prior to test.
E.P.	F41	+16	14.0	22.9	67.0	Nodular goiter; possibly mild thyrotoxicosis clinically.
M.H.	M47	−14	6.07	43.0	30.8	Thyroidectomy in 1945 for thyrotoxicosis; relapse in 1954; propylthiouracil discont'd 3 wks. prior to test; possibly mild thyrotoxicosis clinically.

thyroidism, hyperthyroidism, or normal thyroid function. The average values of the ratios were similar in the three groups although there was a wide individual variation.

The average values for the ratios of salivary I^{131} to plasma-precipitable I^{131} (SI^{131}/PBI^{131}) at twenty-four and forty-eight hours decreased in the order hypothyroid, euthyroid, hyperthyroid.

The salivary I^{131} secretion expressed as a percentage of the dose per liter at twenty-four and forty-eight hours is listed in the last two columns of Table 2. The averages also decreased in the order hypothyroid, euthyroid, hyperthyroid.

In 9 experiments on 7 patients, daily SI^{131}/PI^{131} ratios were obtained for five to ten days, as shown in Table 3.

Data from the 4 patients who were given I^{131}-labeled thyroxine are listed in Table 4. The average SI^{131}/PI^{131} ratios (column 5), the SI^{131}/PBI^{131} ratios, and the salivary secretion were not significantly different from the 48-hour hyperthyroid values (Table 2).

DISCUSSION

The lack of any correlation between the SI^{131}/PI^{131} ratio and thyroid dysfunction is noteworthy. The values in Tables 2 and 3 support and

TABLE 2. RATIOS OF SALIVARY-PLASMA-NONPRECIPITABLE I^{131} AND SALIVARY-PLASMA-PRECIPITABLE I^{131} AND SALIVARY I^{131} SECRETION AT 24 AND 48 HOURS AFTER ADMINISTRATION OF I^{131} AS IODIDE

Patient	Diagnosis	SI^{131}/PI^{131}		SI^{131}/PBI^{131}		Salivary I^{131} (% dose/L.)	
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
W.C.	Hypothyroid	86	50	4,193	1,910	130.0	40.1
W.C.		31.5	76	500	675	47.5	68.8
L.D.	Hypothyroid	21,7	16.2	490	196	12.0	2.0
L.D.		37	38	1,658	402	27.4	6.9
H.B.	Hypothyroid	20	21	294	79	21.2	3.0
N.G.	Hypothyroid	17	20	564	240	15.8	8.4
N.G.		11.6	37.1	300	249	14.1	12.0
	Average	*32*	*36.9*	*1,143*	*536*	*38*	*20*
J.W.	Euthyroid	25	12.5	226	144	13.6	4.0
B.B.	Euthyroid	22	25	508	229	20.3	4.6
B.B.		29	21	950	185	32.3	5.2
C.M.	Euthyroid	16.3	6.2	377	45	13.2	3.6
C.M.		39	29	741	256	40.0	10.3
S.M.	Euthyroid	28	26	325	170	17.2	9.9
S.M.		21.2	50	495	55	33.6	0.4
	Average	*26*	*24*	*517*	*155*	*24*	*5.4*
A.P.	Hyperthyroid	44	9.4	16	3	3.1	0.8
A.P.		58	26.6	42	5	5.4	1.0
R.T.	Hyperthyroid	31	(? 1.7)	36	25	1.4	0.2
O.W.	Hyperthyroid	12.3	5.5	1.4	0.5	1.5	0.7
E.P.	? Hyperthyroid	27	10.5	10.5	8.3	4.3	0.2
M.H.	? Hyperthyroid	33.3	26.7	10.7	3.3	4.5	0.6
	Average	*29*	*16*	*19*	*7.5*	*3.4*	*0.6*

TABLE 3. RATIOS OF SALIVARY-PLASMA-NONPRECIPITABLE I^{131} FOR FIVE TO TEN DAYS AFTER ADMINISTRATION OF I^{131} AS IODIDE
(averages for 3 patients in each group)

Diagnosis	Days after dose of I^{131}									
	1	2	3	4	5	6	7	8	9	10
Hypothyroid	25	25	26	22	19	14	—	—	—	—
Euthyroid	24	30	34	19	21	44	37	29	23	32
Hyperthyroid	34	16	18	30	21	22	21	21	25	18

TABLE 4. RATIOS OF SALIVARY-PLASMA-NONPRECIPITABLE I^{131} AND SALIVARY-PLASMA-PRECIPITABLE I^{131} AND SALIVARY I^{131} SECRETION AT 24 AND 48 HOURS AFTER ADMINISTRATION OF I^{131} AS THYROXINE

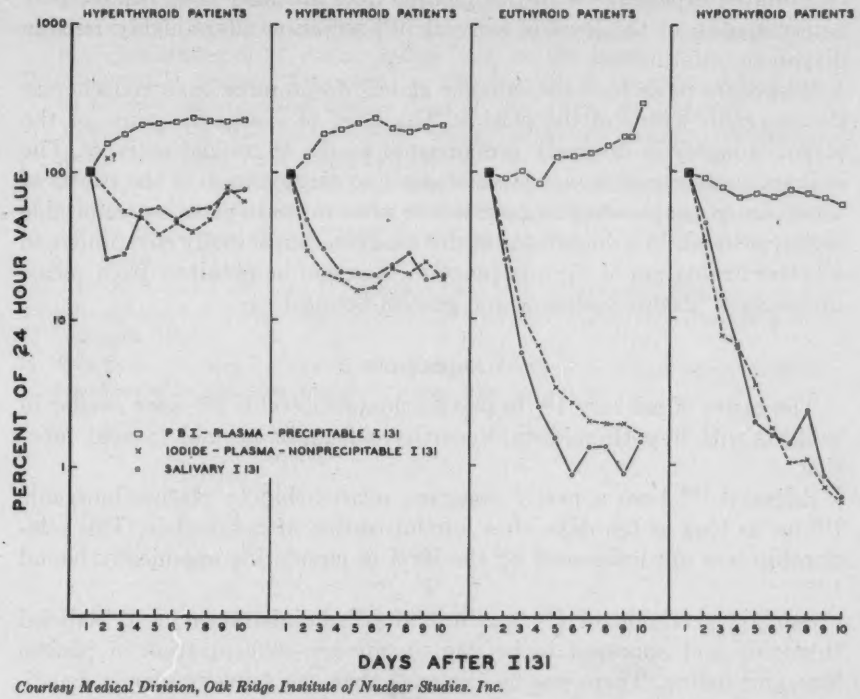
Patient	Dose of thyroxine I^{131} (μc.)	24-Hour ratios		Average	Salivary I^{131} secretion (% dose per L.)	
		SI^{131}/PI^{131}	SI^{131}/PBI^{131}	SI^{131}/PI^{131}	24 hrs.	48 hrs.
E.C.	203	6.7	0.3	25.8 [6]*	4.0	3.8
W.C.	423	18.8	2.2	14.4 [7]	9.4	11.2
S.M.	203	7.1	0.5	14.2 [10]	5.7	9.1
H.H.	203	11.7	0.8	12.0 [10]	4.3	5.0

* The figures in brackets denote the number of consecutive 24-hour ratios included in each average.

extend the earlier report of Freinkel and Ingbar (8), who also found that there was no correlation with thyroid function in patients studied for short intervals after the administration of I^{131}. This similarity of ratios in patients with normal, increased, or decreased thyroid function indicates that the mechanism for salivary concentration of plasma iodide is not affected by the factors that influence thyroidal concentration of iodide in hyperthyroidism and in hypothyroidism. Furthermore, the SI^{131}/PI^{131} ratios were essentially the same whether I^{131} was given as radiothyroxine or as radioiodide. This fact, along with the very low SI^{131}/PBI^{131} ratios after administration of radiothyroxine, suggests that the salivary activity is derived from plasma inorganic iodine. The three SI^{131}/PBI^{131} ratios with values less than 1 can only mean that organic plasma iodine does not diffuse freely through the salivary cells. This finding appears incompatible with the hypothesis of Fawcett and Kirkwood (10, 11) that the salivary glands function "to control the level of thyroxin in the blood stream by deiodinating the hormone and recycling the iodide ion to the thyroid gland via the saliva and gastrointestinal tract."

Thode, Jaimet and Kirkwood (12, 13) report high SI^{131}/PBI^{131} ratios and salivary I^{131} secretion in cases of hypothyroidism, and low SI^{131}/PBI^{131} ratios and salivary I^{131} secretion in cases of hyperthyroidism. This type of correlation of SI^{131}/PBI^{131} with clinical thyroid status is taken as evidence for Fawcett and Kirkwood's hypothesis—an interpretation that depends upon the significance of the SI^{131}/PBI^{131} ratio. These authors determined the salivary-total plasma iodine activity ratios for their patients and found them to be high in hypothyroidism and low in hyperthyroidism. This fact is utilized to support the suggestion that the SI^{131}/PBI^{131} ratios are of more significance than "merely the introduction of a factor proportional to the

radioiodine level in the plasma." The salivary to *total* plasma I^{131} ratio, however, does not reveal the dependence of salivary activity upon plasma inorganic iodide. This dependence is clearly indicated in Figure 1. To demonstrate the relationships, salivary I^{131}, plasma-nonprecipitable I^{131}, and plasma-precipitable I^{131} activities were plotted for periods up to ten days.



Courtesy Medical Division, Oak Ridge Institute of Nuclear Studies, Inc.

FIG. 1. Averages of data on 3 patients in each group, showing the relationships between the changes in salivary I^{131} secretion, plasma iodide, and plasma protein-bound iodine. Note that the ordinate is a log scale. The time scale is in days after the oral administration of I^{131}. The 24-hour value is taken as 100 per cent.

It can be seen that the salivary I^{131} is related to plasma inorganic I^{131} in each of the 4 clinical groups, and changes in plasma PBI^{131} do not affect salivary I^{131} secretion. This relationship also holds in patients who are given radiothyroxine. Thus, if most of the I^{131} activity is circulating as plasma-precipitable iodine, there is a normal SI^{131}/PI^{131} ratio and a low SI^{131}/PBI^{131} ratio with a relatively low salivary I^{131} secretion, regardless of the clinical state of thyroid function. A similar result was also obtained by Freinkel and Ingbar (14). The evidence indicates that the SI^{131}/PBI^{131} ratio is of little or no fundamental significance.

Reference to Table 2 also shows that neither the salivary I^{131} secretion nor the SI^{131}/PBI^{131} ratio was a reliable index of the clinical thyroid status in the hypothyroid group. The 3 hyperthyroid patients were clearly separated from the normal subjects. In 2 patients with questionable hyperthyroidism, the salivary secretion data appeared to support this diagnosis. Our limited experience with this method does not lead us to believe that determination of the level of salivary I^{131} secretion offers highly reliable diagnostic information.

It appears to us that the salivary glands do no more than concentrate the inorganic iodine of the plasma. The level of inorganic iodine of the plasma roughly is inversely proportional to the thyroidial activity. The salivary measurements serve to introduce an exaggeration of the effects of these changes in plasma inorganic iodine when ratios to plasma precipitable iodine are used. It is doubtful that this exaggeration actually contributes to a better evaluation of thyroid function than can be obtained from measurements of plasma inorganic and protein-bound I^{131}.

SUMMARY

The ratios of salivary I^{131} to plasma-nonprecipitable I^{131} were similar in subjects with hypothyroidism, hyperthyroidism, or normal thyroid function.

Salivary I^{131} bore a nearly constant relationship to plasma inorganic I^{131} for as long as ten days after administration of radioiodide. This relationship was not influenced by the level of circulating organically bound I^{131}.

Salivary secretion of I^{131} was low after administration of I^{131}-labeled thyroxine and appeared to be due to salivary concentration of plasma inorganic iodine. There was no evidence that the salivary glands deiodinated thyroxine.

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FURTHER EXPERIENCE WITH THE TOAD METHOD OF TESTING FOR CHORIONIC GONADOTROPIN IN NORMAL AND IN DISTURBED PREGNANCY*

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CURRENT pregnancy testing is largely confined to the diagnosis of pregnancy based upon the qualitative detection of urinary chorionic gonadotropin. However, there is some evidence that in disturbed pregnancies quantitative estimation of chorionic gonadotropin may provide information of prognostic value (1–4). Because such problems are usually of an urgent nature, the rapidly reacting batrachians are well suited for this work providing, a) the seasonal sensitivity to chorionic gonadotropin is known, b) a rapid concentration technique is used in order to rule out false negative reactions, and c) the upper and lower limits of chorionic gonadotropin excretion in normal pregnancy are known.

The most accurate and rapid test in our experience has been a method based on the production of sperm by male toads (1, 5). Male frogs are also satisfactory, providing it is recognized that nonspecific false positive reactions may be observed, especially in the spring (5–7). Although there is considerable clinical advantage in the rapidity with which results can be obtained (two to five hours versus forty-eight to ninety-six hours for the Friedman rabbit and Aschheim-Zondek mouse tests), most batrachians have the disadvantage of being relatively insensitive to chorionic gonadotropin, and are also subject to marked seasonal variation (5, 7–11). The male frog, *Rana pipiens*, is easy to procure throughout the year, but the occurrence of false positive reactions is a handicap. *Bufo americanus* does not have the disadvantage of false positive reactions, but this toad is difficult to obtain except in the spring, and husbandry is a considerable problem (5). The year-round availability of *Bufo marinus*, and an apparently decreased seasonal variation in sensitivity in our studies to date perhaps make it more suitable than the others.

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† Markle Scholar in Medical Science.

The relative insensitivity of toads and frogs requires a concentration technique whenever an injection of unconcentrated urine yields negative results (5, 12). This is necessary in order to eliminate possible false negative reactions. An adsorption technique using kaolin, alumina and buffered urine has been previously reported. This procedure takes about ten minutes and several specimens can be run concurrently (12).

For most clinical purposes it is sufficient to learn if hormone levels are outside the limits of normal. Since most disturbances of pregnancy are abortions and are frequently associated with low titers of chorionic gonadotropin, much information can be gained by merely checking to see if the titer is greater or less than 3000 I.U. and 750 I.U. per twenty-four hours. If 2 animals are used to check each of these levels, the possibility of error is small (1). For the detection of suspected high titers in cases of mole or choriocarcinoma it is necessary to use a larger number of animals or a dose-response curve (1).

In practice, 12-hour urine specimens are used and the aliquot of urine injected varies in accordance with the level being checked and the sensitivity of the animal at that time. If the volume of urine to be injected exceeds 10–15 ml., the concentration technique (12) is used and an allowance made for loss of 50 per cent of the chorionic gonadotropin during this procedure.

This report summarizes three years' experience with the male toads *Bufo americanus* and *Bufo marinus* used for the diagnosis of pregnancy and for prognosis in disturbed pregnancies. Since an extensive evaluation of *Bufo americanus* has been given previously (5), this discussion primarily concerns *Bufo marinus* and minor changes in the rapid concentration technique. The actual techniques for the use of batrachians for pregnancy testing have been described in detail elsewhere (5). The only variation with the male *Bufo marinus* is the use of a 10-ml. injection in place of a 5-ml. injection (as with *Bufo americanus* or *Rana pipiens*) and the need to check finally at five hours instead of four because the reaction is slightly slower in *Bufo marinus*.

METHODS

Bufo marinus

Sensitivity. Because *Bufo americanus* toads were difficult to obtain in the winter months and the husbandry of a large number of experimental animals for long periods of time proved to be a formidable task, an attempt was made to find an animal which was always available and which was indigenous in an area where seasonal climatic changes were minimal. It was felt that minor climatic changes might be reflected in less seasonal variation in sensitivity to chorionic gonadotropin.

Beginning September 1954, 75 to 100 *Bufo marinus* toads weighing 60 to 90 grams each were given injections consisting of graded doses of the International Standard of chorionic gonadotropin or a substandard which was standardized against the International Standard. The percentages of reacting animals were plotted as probits against

the logarithm of the dose and a straight line fitted by the method of least squares (Fig. 1). It was thereby possible to determine the slope of the line (*b*) and the dose of hormone at which 50 per cent of the animals showed positive reactions (E.D. 50).

Although the slope of the line remained fairly constant from month to month, E.D. 50 varied (Table 1 and Fig. 2). However, the variations were smaller with *Bufo marinus* than those previously observed with male *Rana pipiens* or male *Bufo americanus* (5). Sensitivity curves were plotted monthly. Figure 1 is such a curve and shows a calculated E.D. 50 of 23.4 I.U. The limits 19.1 I.U. and 28.6 I.U. have a 95 per cent probability of including the true E.D. 50. In the disturbances of pregnancy in which quantitative

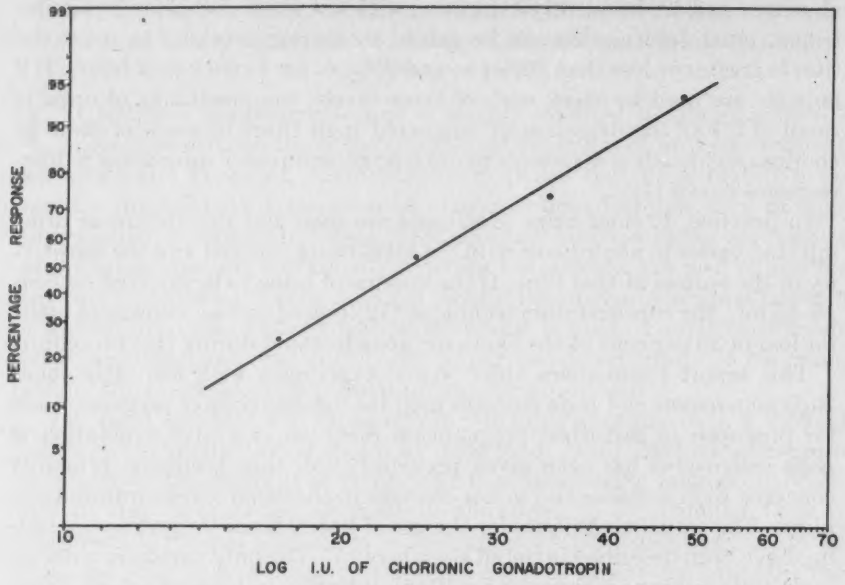

FIG. 1. Response of *Bufo marinus* to graded injections of chorionic gonadotropin (January 1955).

estimations of chorionic gonadotropin need not be exact, it may be possible to select a mean value for E.D. 50 and arbitrarily use this each month instead of actually determining it. This is only feasible if the range over twelve months does not exceed the allowable margin of error in this type of procedure.

From Table 1, the mean value for E.D. 50 is 23.9 I.U. with a standard deviation of ± 3.21 I.U. Thus the 95 per cent confidence limits for E.D. 50 would be 17.5 I.U. and 30.3 I.U. This amount of error does not materially compromise the value of quantitative estimations of chorionic gonadotropin in disturbed pregnancies, since a "less than" value is based upon E.D. 100 (1), and under these prescribed conditions the chances are much greater that the actual level is closer to E.D. 50 than to E.D. 100. This factor and the conservative statement of a "pathologically low level" of chorionic gonadotropin (1) would seem more than adequate to compensate for variation in seasonal sensitivity when *Bufo marinus* is used as the test animal. The results achieved with the use of a mean value for E.D. 50 in actual clinical work will be presented later.

TABLE 1. SEASONAL SENSITIVITY OF *Bufo marinus* TO INJECTIONS OF CHORIONIC GONADOTROPIN

	E.D. 50 (I.U. of CG)	
1954 September	30	
October	23	
November	22	
December	18.5	
1955 January	23.5	
February	21.5	Mean 23.9
March	23	S.D. ±3.21
April	26.5	
May	20.5	
June	25	
July	26.5	
August	27	

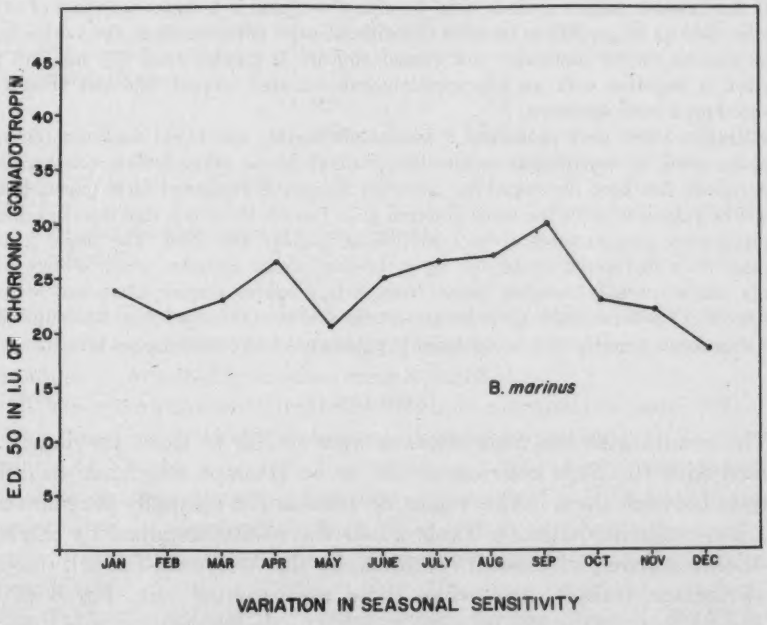

FIG. 2. Seasonal sensitivity of *Bufo marinus* (60–90 grams) to injections of chorionic gonadotropin.

Specificity. In the routine diagnosis of pregnancy, over 1,000 animals received injections of urine from patients who were not pregnant. False positive reactions were noted in 2 animals. Since in each instance these false responses occurred in only 1 of a pair of toads, the result was considered "equivocal" (5). In the repeated test both animals in the pairs showed negative reactions and the correct result was reported.

Negative reactions were occasionally noted in 1 animal of a pair. When this was checked by repetition of the test at the same or an even less sensitive level, 2 positive reactions were obtained. Such sporadic negative findings are probably due to the rare occurrence of a refractory or less sensitive animal, and emphasize the need for reporting conclusive results only when both animals have similar reactions.

Rapid concentration technique

The rapid concentration technique as originally described (12) has continued to be very satisfactory. In 1 instance a false negative result was obtained when the patient was pregnant and subsequently delivered a normal infant. This error may have been related to an attempt to concentrate chorionic gonadotropin (CG) from a very dilute urine (1,550 ml. per twelve hours), since it has been noted in recovery experiments using this concentration technique that the percentile recovery of gonadotropin is less when low concentrations of CG are present (12). In this earlier study it was found that when concentrations of CG were as low as 2 I.U. per ml., the amount of CG recovered was significantly decreased. Thus, for checking a CG level of 3,000 I.U. per twenty-four hours the total volume should not exceed 1,500 ml.

If this possible source of error is to be minimized, and if a direct injection of urine is to be used as frequently as possible (1) without prior concentration, the twelve-hour urine volume should preferably not exceed 500 ml. If greater than 750 ml. and the reaction is negative with an appropriately concentrated aliquot, the test should be repeated on a fresh specimen.

Filtration times were prolonged if particulate matter and blood were not removed from the urine by centrifuging or filtration through coarse paper before concentration.

A funnel[1] has been developed for use with disposable hardened filter paper. If this funnel be substituted for the usual sintered glass funnel, there is a significant saving of filtration time and no need for back-suctioning to clean the filter. The paper is held in place on a perforated lucite disc by a threaded metal cylinder which screws down firmly into a specially designed funnel base. A thin rubber washer interposed between the disc and the filter paper provides an airtight seal between the funnel walls and filter and thus forms a unit which is functionally equivalent to a sintered glass funnel.

RESULTS

The results with the *Bufo marinus* were similar to those previously reported with the *Bufo americanus* (5), so no attempt was made to differentiate between them in this report on tests in 705 normally pregnant and 531 non-pregnant patients. Table 2 lists the results obtained by the toad method compared with results obtained by the Aschheim-Zondek (mouse) or Friedman (rabbit) test when these were carried out. For 6 of the

[1] This funnel is constructed by National Reagents, Main Street, Bridgeport, Connecticut.

705 pregnant patients the reaction was negative in tests performed ten days after a missed period. Thus the accuracy was about 99.5 per cent. When delivery dates were checked in these 6 patients, 5 of them had calculated gestation periods of about eleven lunar months, when based on the first day of the last normal period. Whether these patients represent examples of prolonged gestation, or whether the first missed period was merely a transitory menstrual aberrration in an otherwise normal menstrual history, is not known. The remaining patient was definitely pregnant at

TABLE 2. COMPARISON OF TOAD METHOD WITH ASCHHEIM-ZONDEK AND FRIEDMAN TESTS FOR PREGNANCY DIAGNOSIS

	No. of subjects	Toad method		Friedman (rabbit)		A-Z (mouse)	
		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Normal pregnant women (ten days after first missed period)	145	145	0	145	0	—	—
	128	128	0	—*	—	127	1
	432	426	6	—	—	—	—
Totals	*705*	*699*	*6†*	*145*	*0*	*127*	*1*
Non-pregnant women	71	0	71	0	71	—	—
	192	0	192	—	—	2	190
	268	0	268	—	—	—	—
Totals	*531*	*0*	*531*	*0*	*71*	*2*	*190*

* Dash signifies that test was not performed.
† *See* text.

the time of the test and the error was probably related either to a very dilute urine specimen (as discussed previously under "Rapid concentration technique"), or possibly to some error in technique.

Of the quantitative estimations of chorionic gonadotropin (CG), 127 were carried out with *Bufo marinus* using an approximate E.D. 50 of 25 I.U. of CG (*i.e.*, an E.D. 100 of about 50 I.U.). The results were essentially the same as those computed from the actual monthly E.D. 50. This would suggest that an approximate E.D. 50 of 25 I.U. of CG for the male *Bufo marinus* may be adequate for quantitative estimation of CG in disturbed pregnancies.

In Table 3 are listed the results of tests for the presence of CG in the urine of 347 patients whose pregnancies terminated in abortion. This group included only those cases in which there was doubt about the clinical

outcome; therefore it was largely made up of cases clinically diagnosed as "threatened abortion." In 212, or about 61 per cent of this group of "threatened abortions," the titer of CG was less than 3000 I.U. per twenty-four hours, indicating that the fetus was lost, although this condition was not clinically evident. Figure 3 illustrates this point. Of the 116 Aschheim-Zondek (A-Z) monitored cases with titers of CG less than the pathologically low level of 3000 I.U. per twenty-four hours (1), 50 showed positive A–Z reactions which were prognostically misleading. Thirty-nine per cent of this group had titers which were not considered abnormal. It should be

TABLE 3. ESTIMATION OF URINARY CHORIONIC GONADOTROPIN IN PREGNANCIES WHICH TERMINATED IN ABORTION—TOAD METHOD, MONITORED BY ASCHHEIM-ZONDEK METHOD

	No. of cases	Toad method (more than 3000 I.U. of CG)	A-Z (mouse)		Toad method (less than 3000 I.U. of CG)	A-Z (mouse)	
			Pos.	Neg.		Pos.	Neg.
Abortions	282	116	39	2	166	39	37
Ectopic pregnancy	35	14	4	1	21	7	9
Missed abortions	30	5	5	0	25	4	20
Totals	*347*	*135**	*48*	*3*	*212**	*50*	*66*

* Some tests were not monitored with A-Z mouse tests.

noted that many patients with normal titers may eventually abort from a non-hormonal cause; or in cases of inevitable abortion, a considerable amount of remaining chorionic tissue may maintain "normal" levels of CG.

COMMENT

Further experience with the toad method of pregnancy testing indicates that it is accurate for the diagnosis of pregnancy and that a pathologically low level (3,000 I.U.) of urinary chorionic gonadotropin per twenty-four hours is a valuable guide in the disturbances of pregnancy. Certain precautions must be observed when quantitative estimations are performed in cases of probable disturbed pregnancy. Accurately timed specimens are required, and preferably the total volume of a twelve-hour specimen should be less than 500–750 ml. If the specimen is not clear, it must be filtered through coarse filter paper. Unless these simple rules are observed, errors may result (12).

Date of last normal menstrual period. The prognostic value of assay of urinary gonadotropin in disturbances of pregnancy is predicated upon the detection of a pathologically low level of CG in the period from the seventh

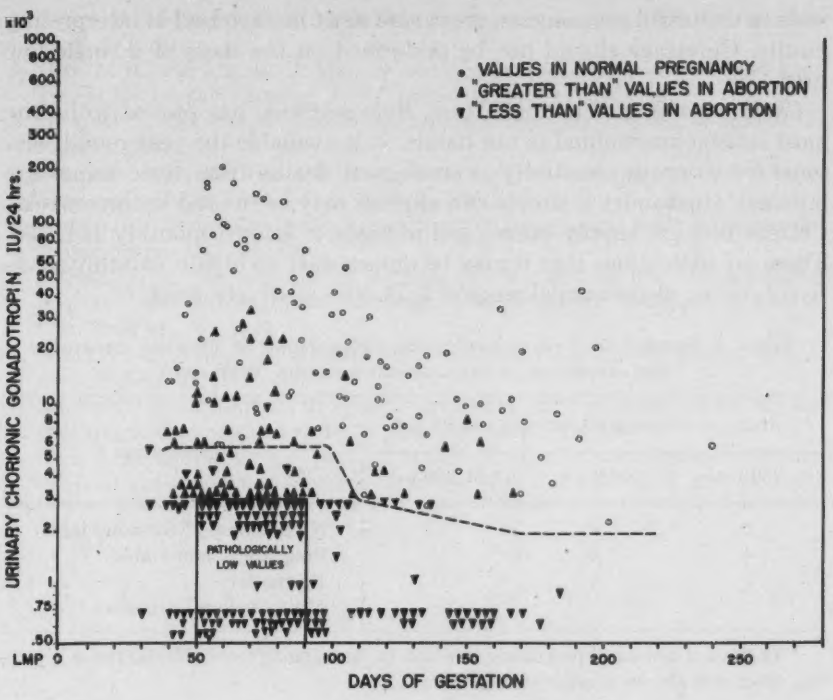

FIG. 3. Estimations of chorionic gonadotropin in abortion are shown as "greater or less than" values. The values in "normal pregnancy" are actual ones within the limitation of the bioassay method employed.

to the thirteenth week of gestation (1). It is therefore necessary to establish correctly the time of the last normal menstrual period. When the cycles have been irregular, the actual time of conception may have been weeks or even months from that calculated on the basis of the last menstrual period. In this limited series of 580 patients there were 5 such women, or roughly 1 in 100. Statistically, 1 in 5 of all pregnant patients experiences vaginal bleeding during the course of pregnancy. Theoretically then, there would be a chance that bleeding with menstrual aberration would occur approximately once in 500 pregnancies.

Interpretation of laboratory findings. The clinical status of the patient can be assessed only if laboratory findings and clinical findings are considered together. Table 4 is a guide to the conclusions that may be drawn from various estimations of chorionic gonadotropin. A 500,000 I.U. level of CG is checked if a mole or choriocarcinoma is clinically suspected.

Although quantitative determination of gonadotropin is useful for prog-

nosis in disturbed pregnancies, great care must be exercised in interpreting results. Curettage should not be performed on the basis of a single low titer.

Choice of animal. The male toad, *Bufo marinus*, has proved to be the most satisfactory animal in our hands. It is available the year round, seasonal variation in sensitivity is small, and deaths from toxic urines are minimal. Husbandry is simple and animals may be re-used in three weeks. Certain biologic supply houses sell animals of known monthly E.D. 50. There are indications that it may be unnecessary to obtain monthly sensitivity curves, as the annual range of E.D. 50 is relatively small.

TABLE 4. SIGNIFICANCE OF QUANTITATIVE ESTIMATIONS OF URINARY CHORIONIC GONADOTROPIN IN THE GESTATION PERIOD 50–90 DAYS

Urinary chorionic gonadotropin per 24 hrs.			Interpretation
750 I.U.	3000 I.U.	500,000 I.U.	
0	0		No pregnancy* (or non-viable)
+	0		Pregnancy; non-viable
	+		Pregnancy
		+	Mole or choriocarcinoma

* In cases of disturbed pregnancy in which no functioning trophoblastic tissue is present, there will also be a negative reaction.

SUMMARY

1. The male toad, *Bufo marinus*, appears to be a satisfactory animal for use in tests to establish the diagnosis of pregnancy and for quantitative estimations of urinary chorionic gonadotropin in the disturbances of pregnancy.

2. Availability, minimal seasonal variation in sensitivity, and easy husbandry suggest that it is more suitable as a pregnancy-test animal than the batrachians previously studied.

3. Using this toad in the assays, further studies of the quantitative estimation of urinary chorionic gonadotropin confirm its prognostic value in disturbed pregnancies.

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FULMINATING ADRENOCORTICAL HYPERFUNCTION ASSOCIATED WITH ISLET-CELL CARCINOMA OF THE PANCREAS: CASE REPORT*

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THE association of adrenocortical hyperfunction and carcinoma of the pancreas is not coincidental and is of considerable interest. In a review (1) of 114 autopsies of patients with Cushing's syndrome, the incidence of neoplasms *other* than those involving the adrenal or pituitary was reported as 10 per cent. There were 4 cases of pancreatic carcinoma.

Crooke (2) reviewed these 4 cases of pancreatic carcinoma associated with basophilism. In the first case an adrenal tumor was removed surgically, but it was a metastasis from a carcinoma of the head of the pancreas that was found at autopsy. Full histologic details are not available. The second case was probably an acinar-cell carcinoma of the pancreas, although the authors believed that the tumor of the panereas was derived from an adrenocortical rest because its cells contained granules which took an acidophil stain by Vines' method; Crooke points out, however, that the zymogen granules of normal pancreatic cells also take this stain, and it is possible that this tumor was a primary pancreatic carcinoma. The third case was that of a 50-year-old woman with typical Cushing's disease, but no detailed examination of the pancreatic tumor was reported. The fourth case was Crooke's—a patient who had a scirrhous carcinoma of the pancreas composed of abundant, dense collagenous tissue, embedded in which were cells which resembled in their staining the cells of the pancreatic acini.

Del Castillo *et al.* (3) in 1950 reported the first islet-cell carcinoma of the pancreas to be associated with Cushing's syndrome. His patient was a 38-year-old Argentinian woman in whom a pancreatic lesion was suspected, but not proved before death, which occurred eight months after admission to the hospital. The case reported here is, therefore, the second in which an islet-cell carcinoma of the pancreas occurred in association with Cushing's syndrome, and it is the first of the cases discussed in which an attempt was made to remove the pancreatic tumor at operation.

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The most striking features about our patient and the 5 reported in the literature were: 1) that only 1 was as old as 50 years; among the other 5, 3 were between 20 and 30 years old, 1 was 38 and 1 was 40—ages at which carcinoma of the pancreas is rare; and 2) that in all, the disease was acute in onset and rapid in progress.

CASE REPORT

History

A 40-year-old white woman was admitted to All Souls Hospital, Morristown, N. J., on August 11, 1954, complaining of facial hirsutism and generalized swelling of three weeks' duration. On July 22, 1954, because of headache and dysuria, she visited an osteopathic physician, who found generalized edema, and noted an occasional pus cell and some bacteria in her urine. He prescribed an antibiotic and ammonium chloride. A week later he gave her an injection of 2 milliliters of Mercuhydrin®, which resulted in a diuresis of about a gallon.

Pertinent facts on admission to the hospital were as follows:

Skin: Rounding of the face with puffiness of the eyelids and swelling of the legs had been recently noted. An excessive growth of fine hair had appeared on the upper lip and sides of the face within the previous three weeks. The skin had become oily and a few pustules had erupted on the face and chest. Easy bruisability and thinning of the skin were noted, and the nails had become brittle. About two weeks before admission, a black spot about 1 millimeter in diameter had developed on her right index finger. For five nights before admission sweating had been severe, but only from the waist up. A fungus infection about the anus had been present in early 1954, and had healed with great difficulty.

Central nervous system: Crying had recently been excessive. Sleepiness had been present for the previous three weeks and a throbbing headache for the previous two weeks.

Special senses: Everything tasted salty and there had been a craving for sweets.

Cardiovascular: There had been no dyspnea or orthopnea. The blood pressure was 128/78 mm. Hg on July 29, 1954 and 150/80 on August 10, 1954.

Gastro-intestinal: Appetite was greater than usual. Stools were watery and had recently averaged four per day.

Genito-urinary: Menses had been regular every twenty-six days until six months before, at which time a thirty-day cycle had become established with the exception of one seven-week interval. She had been pregnant twice, having 1 living 8-year-old child and 1 miscarriage. Nocturia had occurred twice in the period shortly before entry.

Musculo-skeletal: No muscular weakness had been noted. Aching in the lumbo-sacral region had been present for the previous three weeks, but such aching had been intermittently present since a back injury in 1942.

Physical examination

The patient was a well developed and nourished white woman, appearing about her stated age of 40 years (Fig. 1a). There was no dyspnea, orthopnea, or cyanosis. She did not appear acutely ill, but demonstrated moderate anxiety and depression. The temperature was 98° F., pulse 80, and respirations 16 per minute. Blood pressure was 148/80 mm. Hg.

Main findings were as follows: the face was ruddy and rounded with excessive fine hair on the upper lip and sides of the face (Fig. 1b). The axillary and pubic hair had a normal distribution. *There were no striae.* The skin otherwise was as described in the history. The retinal vessels showed slight A-V compression; the optic discs were not swollen or atrophic. The thyroid gland was symmetrical and slightly enlarged. Examination of the heart, lungs, abdomen, pelvic organs, and genitalia revealed no significant abnormalities. There was 1+ edema of the legs. No muscular weakness was evident.

FIG. 1. Photographs of patient on August 22, 1954.

a. Normal body configuration, hirsutism of upper lip, and absence of obesity and striae.

b. Marked hirsutism of the upper lip and face, slight rounding of the face and thickening of the neck.

Laboratory findings

Preoperative studies carried out at All Souls Hospital and Presbyterian Hospital are summarized in Table 1.

X-ray examinations

Skull and chest films were normal. Spinal studies indicated questionable demineralization. In an intravenous pyelogram there was a suggestion of a dense area overlying the upper pole of the right kidney. Retrograde pyelography showed the same density. Presacral air studies then revealed a normal left kidney and adrenal gland; the right kidney shadow appeared normal, but there seemed to be a mass overlying the upper third of the right kidney; this was interpreted as a possible adrenal tumor.

TABLE 1. PREOPERATIVE LABORATORY FINDINGS

Procedure	Aug. 12, 1954—All Souls Hosp., Morristown		Aug. 29, 1954—Presbyterian Hosp., New York
Urine Culture	E. coli and Staphylococcus albus. No acid-fast bacilli. Negative results with guinea-pig inoculation.		—
White Blood Count Neutrophils Lymphocytes	12,500 84% 16%		14,400 96% 4%
Eosinophil Count	0		—
Sedimentation Rate (Westergren)	44 mm. in 1 hr.		—
Glucose Tolerance Test	*Blood* (mg./100 ml.)	*Urine*	*Blood*
Fasting blood sugar	115	0	127 mg./100 ml.
½ hr. after 100 Gm. of glucose orally	204	0	
1 hr. after glucose	224	0	
2 hrs. after glucose	269	2+	
3 hrs. after glucose	279	4+	
Total Plasma Proteins Albumin Globulin A/G ratio	5.5 Gm.% 3.9 Gm.% 1.65 Gm.% 2.3:1		—
CO_2 Capacity	29.4 mEq./L		More than 34.7 mEq./L
Serum Sodium	143.5 mEq./L		148.5 mEq./L
Serum Chloride	87.3 mEq./L		81 mEq./L
Serum Potassium	3.2 mEq./L		1.8 mEq./L
Urinary 17-Ketosteroids	37 mg. per 24 hrs. (Norumburski method; normal 5–15 mg. in 24 hrs.)		39–47 mg. per 24 hrs.; no decrease following i.v. hydrocortisone
Plasma Corticoids	—		92 gamma per 100 ml. (Wallace & Christy method; normal 4–32 gamma per 100 ml.)
Urinary Corticoids	—		41–48 mg. per 24 hrs. (Porter-Silber method; normal 2–6 mg. in 24 hrs.)
Electrocardiogram	Normal		Left ventricular hypertrophy, or digitalis effect, or both

The following determinations yielded results within the normal range: red blood cell count and hemoglobin, urinalysis, serum calcium and phosphorus, blood urea nitrogen, alkaline phosphatase, cephalin flocculation, and basal metabolic rate.

Course

The patient spent ten days at All Souls Hospital while investigations were proceeding. She had spells of weeping and depression during the latter part of her stay. The edema subsided when she received a salt-free diet. There was a reduction in weight from 125

pounds on admission to 119 pounds on discharge. Another small black spot developed on the right third finger while she was under observation.

The patient was discharged from All Souls Hospital on August 20, 1954. On August 29, 1954 she was admitted to the service of Dr. George F. Cahill at the Presbyterian Hospital in New York City.

Dr. Cahill thought the patient had acute Cushing's syndrome, and that the laboratory evidence favored adrenal carcinoma.

She was given a regular diet and continued to have 3+ to 4+ sugar in the urine. No insulin was added. On September 6 she became psychotic and had suicidal tendencies. Her weight increased from 115 pounds on August 29 to 125 pounds on September 12. She was given an injection of Mercuhydrin® and 200 milligrams of cortisone on September 12. There was a loss of 18 pounds by the next day.

Operation

On September 13, through a transverse abdominal incision, Dr. Cahill unexpectedly discovered and removed an orange-sized tumor from the midportion of the pancreas. Both adrenals felt normal at operation. No surface metastases were seen on the liver, but irregular, firm areas were felt.

Postoperative period

Postoperatively cortisone and desoxycorticosterone were continued in gradually decreasing doses; maintenance doses of streptomycin, penicillin, and digitoxin were also administered. On the first postoperative day her temperature rose to 104° F., falling a little on succeeding days. Her abdomen became distended and bowel sounds were hypoactive. By the fourth postoperative day she was disoriented. Severe hypotension developed and she lapsed into coma. Hydrocortisone and noradrenaline were infused, but the temperature rose to 105° F. in spite of apparently clear lungs and the absence of localizing signs. The patient died on the fifth postoperative day (September 18, 1954), approximately two months from the time she perceived the first symptoms.

Postoperative laboratory findings are summarized in Table 2.

Autopsy report

Unfortunately, permission for examination of the pituitary and brain could not be obtained and the autopsy was performed entirely through the abdominal incisional wound by Dr. D. Benninghoff, Presbyterian Hospital, New York. The main findings were as follows:

Pancreas: On exploring the pancreas *in situ*, about 20 ml. of necrotic material exuded from the site of the body of the pancreas, which appeared to have been surgically excised. The head and tail of the pancreas were intact, yellowish-brown in color, and firm in consistency with no evidence of tumor. At the site of the excised tissue there was considerable reddening and inflammation; there were fibrinous adhesions to the pancreatic surface of the stomach in that location.

Microscopic examination (Fig. 2) revealed pancreatic tissue, portions of which had been replaced by atypical cells arranged in nests and cords surrounded by dense connective tissue septa and resembling islet cells. The individual cells possessed irregular round or oval vesicular nuclei; the cytoplasm was acidophilic, granular, and sparse; the cell membranes were indistinct. Numerous vacuole formations were observed.

In some areas fibrosis and hyalinization had occurred, and the tumor cells were seen

TABLE 2. POSTOPERATIVE LABORATORY FINDINGS

Procedure	Days after operation		
	0	3	4
Serum Sodium	142.0 mEq./L	135.0 mEq./L	147.0 mEq./L
Serum Potassium	1.8 mEq./L	2.5 mEq./L	3.6 mEq./L
Serum Chloride	—	84.0 mEq./L	96.0 mEq./L
CO_2 Capacity	—	More than 34.7 mEq./L	18.0 mEq./L
Blood Sugar	—	434.0 mg./100 ml.	—
Electrocardiogram	—	Suggestion of hypokaliemia	—

to surround blood vessels and nerve bundles. Other foci showed infiltration by strands and cords of atypical cells which were more undifferentiated. These anaplastic cells had small spindle-shaped, hyperchromatic nuclei. Mitotic figures were moderate in number.

Adrenals: The left adrenal weighed 16.5 grams and the right 21.0 grams. Although these organs were grossly enlarged, the cortical zone did not appear to be significantly increased in size. There was no gross evidence of localized lesions in the adrenal glands and on cut section the golden-yellow brown cortex was well demarcated from the whitish medulla. Examination of the left adrenal was not completely satisfactory because considerable autolysis and maceration had taken place in removing it.

Microscopic examination revealed hyperplasia of the cortical elements of both

FIG. 2. Photomicrograph of section from carcinoma of the pancreas. Note arrangement of tumor cells in islet form (H & E; ×45).

adrenals, the zona fasciculata being particularly prominent; numberous foam cells were noted. No tumor was present.

Liver: The liver was enlarged, extending 8 centimeters below the costal margin and weighing 1,600 grams. On its surface there were several small whitish nodular areas surrounded by a zone of purplish discoloration. On palpation, these areas were very firm in consistency. On cut section the liver was found to have several of these greyish-white nodules throughout its parenchyma, the largest measuring 5.5 centimeters in diameter. Most of the other nodules were 5–10 millimeters in diameter. The remainder of the liver was pale tan in color and firm in consistency.

Microscopic examination revealed moderate fatty metamorphosis, largely centrilobular in location, along with focal central necrosis and a considerable amount of glycogen vacuolization of the hepatic cells. Tumor was present in two of the sections and its cells were arranged as masses of anastomosing cords. Nuclei were for the most part large, hyperchromatic, and ovoid or spheroid in shape; their cytoplasm was eosinophilic and the tumor cells resembled those of the islets of Langerhans.

Heart: This organ weighed 275 grams and was unremarkable grossly and microscopically.

Aorta: Scanty atheromatous changes were present in the portion of abdominal aorta examined.

Lungs: The lungs were crepitant throughout. Congestion of the septal vessels and foci of atelectasis were evident. There were red cells and macrophages containing yellow-brown pigment in the alveoli.

Pathologic diagnosis

1. Islet-cell carcinoma of the body of the pancreas with metastases to the liver.
2. Bilateral adrenal hyperplasia.
3. Postoperative acute pancreatitis.

COMMENT

Evidence for the conclusion that the patient was suffering from an adrenal carcinoma was the shadow overlying the right kidney, observed in all x-ray studies, and the failure of the urinary 17-ketosteroid level to fall after the administration of intravenous hydrocortisone. The mass seen on x-ray examination remained unexplained, both at operation and at autopsy. Cahill (4) has stated that the interpretation of perirenal air insufflation films of patients with Cushing's syndrome is particularly difficult because of the "edema in the perirenal tissue" in this condition.

Since adrenal hyperfunction was obviously present and an adrenal lesion suspected, an attempt was made to determine whether it was benign hyperplasia or a tumor, by means of the intravenous hydrocortisone test as proposed by Jailer (5). The test results were considered negative, indicating the presence of a tumor which was autonomous in behavior and situated either in the pituitary or the adrenal gland. That it was not in the adrenal was proved at laparotomy. Whether or not it was in the pituitary unfortunately could not be investigated because of the restrictions imposed

on the autopsy. However, if one considers this test valid, the conclusion is that the patient was suffering from a pituitary tumor which induced the adrenal hyperplasia.

The patient's symptoms and signs differed from those usually associated with Cushing's disease, in that she was not obese, there were no striae, the blood pressure and red blood count were normal, and menstruation had continued more or less regularly. In all other respects the preoperative laboratory findings were in accord with those usually observed in adrenocortical hyperfunction or following prolonged treatment with corticotropin or cortisone. Characteristic of this condition were the leukocytosis with lymphopenia and absence of eosinophils, the diabetic-type glucose tolerance curve, decreased level of plasma proteins, hypochloremic alkalosis, low level of serum potassium, increased urinary 17-ketosteroids, and increased plasma and urinary corticoids. An elevated sedimentation rate has not been described in adrenocortical hyperfunction; the elevated rate in our case may have been due to the pancreatic carcinoma. The differences noted between our findings and those in Cushing's syndrome may well have been due to the accelerated course of the disease in this patient.

Neither in this case nor in del Castillo's (3) was there a history of hypoglycemic attacks; both patients, in fact, had a glucose tolerance curve of the diabetic type. This is not surprising, however, since only a minority of islet-cell tumors are functional. Melicow (6) reviewed the autopsy records of 4 patients with islet-cell tumors of the pancreas, and in none was there any significant finding in the adrenals.

The postoperative course, metabolically, was characterized mainly by the persistent hypopotassemia, hyperglycemia and hypochloremic alkalosis. It is interesting, but unexplained, that the carbon dioxide capacity dropped from more than 34.7 mEq./L to 18 mEq./L within the twenty-four hours preceding death. It was felt that the acute pancreatitis around the area of excision was probably sufficient to tip the balance in this case, since patients with Cushing's syndrome are known to tolerate postoperative inflammation or infection very poorly. Moreover, this patient was treated with gradually decreasing doses of cortisone and desoxycorticosterone postoperatively. This would tend to enhance the already existent adrenocortical hyperfunction, since the adrenals were not excised at operation and a test dose of intravenous hydrocortisone had caused no decrease in production of 17-ketosteroids. Thus it was indicated that any cortisone administered to this patient would augment the endogenous supply rather than decrease its production in the normal manner.

When Cushing's syndrome is associated with a tumor, there is always the question of whether the neoplasm causes the hyperadrenalism or

whether the latter causes or encourages the growth of the neoplasm. M. G. Thorne (7) and others have discussed the various relationships but as yet there is no definite answer. Thorne concludes "it is possible that the aetiological relationship is more subtle; that an innate abnormality of adrenal cortical metabolism destined in later life to give rise to malignant disease, is precociously awakened in Cushing's syndrome, possibly as a result of excessive production of adrenocorticotrophic hormone. This would be compatible with the fact that the neoplasms here discussed occurred chiefly in young subjects and with the conception, previously expressed by Findley (1949), that Cushing's syndrome affords a 'possible clue to certain disorders associated with ageing'."

In only 1 other instance was an attempt made to remove the tumor—a thymic carcinoma, in a patient reported by Hubble (8). In this patient death occurred twenty-four hours after operation, too soon to determine what effect such removal would have on the hyperadrenalism. In our case, death occurred five days after operation—also too soon to determine the effect of removal of an islet-cell carcinoma of the pancreas on the symptoms of hyperadrenalism.

SUMMARY

1. The second case of hyperadrenocorticism (Cushing's syndrome) associated with islet-cell carcinoma of the pancreas has been presented.

2. Five cases of Cushing's syndrome associated with carcinoma of the pancreas have been previously reported. This sixth case of adrenocortical hyperfunction is the only one in which an attempt was made to remove the pancreatic tumor.

3. The use of the intravenous hydrocortisone test in the differential diagnosis of adrenal hyperplasia and pituitary or adrenal tumor is discussed.

4. The possible relationship of adrenal hyperplasia to carcinogenesis is noted.

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THE SYNDROME OF FAMILIAL JUVENILE HYPOADRENOCORTICISM, HYPOPARATHYROIDISM AND SUPERFICIAL MONILIASIS

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INTRODUCTION

THE coincidence of hypoparathyroidism and Addison's disease in children is rare, only 7 cases being found in the available literature (1–9). The present paper surveys this material briefly and presents an additional patient with hypoadrenocorticism and hypoparathyroidism—a 15-year-old boy studied at The Cincinnati Children's Hospital, in whom the clinical diagnoses were confirmed by autopsy findings. From these data, evidence indicating that the syndrome in question has in general the features of "familial juvenile hypoadrenocorticism, hypoparathyrodism and superficial moniliasis" has been assembled, and the suggestion offered that other reported cases with some of these features are incomplete examples of the syndrome. Relationships between the monilial infection and the endocrine dysfunctions, and other implications of this syndrome, are also discussed.

CASE REPORT

N.A. (#93544). This white boy was admitted to The Cincinnati Children's Hospital in February 1954, at the age of $15\frac{3}{12}$ years, with a history of lassitude and progressive generalized weakness associated with occasional episodes of vomiting, of three months' duration. Little past and family history was available. Because of separation of the parents and family financial difficulties, this patient and his siblings were institutionalized at an early age. The health of the parents, of 3 older brothers, and of 1 older sister was reported as not abnormal. The patient entered a custodial institution at the age of 2 years. He was described at that time as a "puny child who had funny fingernails and white patches in the mouth." His growth and development were somewhat retarded, and he did poorly in school. He was unable to get along with school children his own age, and was more congenial with younger children. He was ostentatious and unreliable, and was considered a "continuous trouble maker." Although never very active, he did enter into some outdoor sports.

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Three months prior to admission to this hospital generalized weakness developed and gradually progressed to such degree that he was severely fatigued by short periods of activity or standing. He vomited intermittently and also had episodes of dizziness and syncope, which could be relieved by lying down. There was no history of salt craving, abnormal water balance, elevated temperature, headache, convulsions, tetany, muscle cramps, or symptoms related to other organs during the interval before admission. At his local hospital the diagnosis of Addison's disease was considered, but several diagnostic tests yielded inconclusive results; finally the diagnosis of postural hypotension was made. He was referred to The Children's Hospital for further study.

FIG. 1. The patient at age 15, showing poor general and sexual development, generalized hyperpigmentation, numerous pigmented nevi, and almost complete lack of pubic hair.

First admission

Physical examination revealed a cooperative talkative white boy of average intelligence, who became dizzy upon standing. He had a gaunt face but was otherwise moderately well nourished and developed. His height was 61.5 inches (normal, 60–65 inches) and weight, 82 pounds (normal, 89–113 pounds). The skin showed a definite yellowish or bronze color, with darker zones around the eyes and over the joints. There were many dark pigmented nevi scattered over the entire body, as well as pigmented scars beneath the chin and on the shins. The hair of the legs, arms, eyebrows, axillae and genital region was sparse, and the hair of the scalp was fine, dry and sparse (Fig. 1). With the exception of two normal toenails (Fig. 2), all nails had irregular, rough, crumbled outer edges with hypertrophied bases. The nails were also short, tough, and difficult to cut. The mucous membranes of the mouth and the gums were hyperemic and covered with thick white plaques which resembled those of monilia infection. The tongue was red and smooth with a decreased number of papillae. There were several carious teeth, and all teeth had a chalky appearance, with transverse ridges on the buccal surfaces. No teeth were missing. There was normal sweating. The thyroid was not palpable, and the superficial lymph nodes were not remarkable. Funduscopic examination of the eyes yielded negative results, and there was no evidence of cataract or photophobia. Trousseau and Chvostek signs could not be elicited. Both testes were descended, and of normal consistency, but each measured only 1.5×1 cm.; the penis measured 3 cm. When the patient was lying down his blood pressure was 110/70 mm. Hg; after standing for ten to fifteen seconds, it was 88/50.

Laboratory data were as follows: Hemoglobin, 9.2 Gm. per 100 ml.; red blood cells, 3,010,000 per cu. mm.; white blood cells, 4,900 per cu. mm.; and differential white cell count, normal. The basal eosinophil count was 50 per cu. mm.; four hours after 20 mg. of corticotropin (ACTH) it was 99 per cu. mm. The concentrations of various constituents of the serum were: sodium 130 mEq., potassium 5.8 mEq., chlorides 93 mEq.,

magnesium 1.3 mEq., and CO_2 14.6 mEq. per liter; nonprotein nitrogen 41 mg., cholesterol 284 mg., calcium 10.1 mg., phosphorus 6.3 mg.; alkaline phosphatase 1.9 Bodansky units, carotene 341 μg., protein-bound iodine 6 μg.; total protein 8.5 Gm., albumin 4.6 Gm., and globulin 3.9 Gm. per 100 ml. Results of a glucose tolerance test were as follows: fasting blood sugar 120 mg.; half hour, 233 mg.; one hour, 160 mg.; two hours, 102 mg.; and three hours, 103 mg. per 100 ml. There was no sugar or acetone in the urine, and the pituitary follicle-stimulating hormone content was less than 3 mouse uterine units per twenty-four hours. Urinary 17-ketosteroids were 5.9 mg. per twenty-four hours.

FIG. 2. The patient's hands and feet, showing monilial lesions of all nails save one toenail on each foot. Note the numerous pigmented nevi.

A roentgenogram of the chest showed that the cardiac shadow was markedly decreased in size. The lung fields were clear. In a flat film of the abdomen there was no evidence of calcification in the region of the adrenals. Roentgenograms of the skull showed prominent convolutional markings. Skeletal maturation was normal. The electrocardiogram was within normal limits.

Culture of the nails produced Candida albicans.

Hospital course. The patient became progressively weaker. Because of the clinical data, and the chemical findings of hyponatremia and hypochloremia, the patient was first treated with a diet containing 15 grams of salt per day. He showed marked improvement within one day, but his serum sodium concentration continued to fall until it was discovered that the patient was discarding much of the salt ration. He was then treated with 2 mg. of desoxycorticosterone acetate (DCA) daily intramuscularly, and again there was improvement in the clinical condition and the chemical findings. When the DCA was discontinued, there was deterioration in both clinical and chemical states. The patient was then given 1 mg. of DCA daily and 5 mg. of cortisone twice daily intramuscularly, and added oral salt. After one month in the hospital he was discharged on

this regimen, to be followed closely in the endocrine clinic. At this time he was gaining weight and was quite active.

Interval

The patient was maintained satisfactorily and he engaged in normal adolescent activities, such as baseball, until May 24, 1954, when it was noted that he had lost weight. Because of doubt as to the actual amount of medication being taken, he was given 25 mg. of desoxycorticosterone trimethylacetate. He returned to the clinic on June 21, 1954, feeling tired, lethargic, nauseated, and having lost 12 pounds. He was admitted to the hospital at this time with the diagnosis of impending adrenal crisis.

Second admission

Laboratory data were as follows: Hemogram and urinalysis—normal findings. Serum concentrations were: sodium 150 mEq., potassium 4.6 mEq., chlorides 96 mEq., and CO_2 27 mEq. per liter; nonprotein nitrogen 40 mg., cholesterol 216 mg., calcium 11.9 and 9.5 mg., phosphorus 4.5 and 5.1 mg., alkaline phosphatase 4.2 Bodansky units, protein-bound iodine 3 μg., total protein 6.9 Gm., albumin 3.4 Gm., and globulin 3.5 Gm. per 100 ml. The cephalin flocculation test yielded negative results. The basal matabolic rate was −20 per cent. Urinary pituitary follicle-stimulating hormone was less than 3 mouse uterine units per twenty-four hours. Urinary 17-ketosteroids were 2.9 mg. per twenty-four hours.

Roentgenograms of the long bones showed diffuse osteoporosis. A flat film of the abdomen showed no calcification in the region of the adrenals and no evidence of renal calculus. In intravenous pyelograms the urinary tract appeared normal. In electrocardiograms on two occasions the QTc was prolonged to 0.45 and 0.47 seconds. These findings were interpreted by the cardiologist as being consistent with hypocalcemia.

Candida stellatoides grew from culture material taken from the nail beds and buccal mucosa.

Hospital course. The patient improved greatly with administration of DCA, cortisone, and intravenous sodium chloride. Physical examination showed little change from the findings at the time of the first admission, except that there was a definite area of alopecia on the left side of the scalp, and the pigmentation of his skin seemed less intense. After the initial treatment the patient was much stronger, and became an almost impossible behavior problem, refusing medication, being destructive to hospital furniture, and being particularly belligerent to nurses and doctors. On August 9, 1954, two 125-mg. DCA pellets were inserted without difficulty into the left scapular region. Before the procedure, some respiratory difficulty developed after he received $\frac{1}{8}$ grain of morphine preoperatively, but these symptoms subsided quickly. The symptoms of Addison's disease had definitely improved. However, the monilia infection of the nails and mouth, the alopecia, and the ridged teeth remained unexplained.

The serum calcium and phosphorus levels remained normal until August 12, 1954 (six months after the diagnosis of Addison's disease), when they were reported as 7.4 mg. and 5.8 mg. per 100 ml., respectively. Within one week definite bilateral Chvostek signs developed. The serum phosphorus level rose to 7.1 mg. and the calcium level dropped to 6.8 mg. per 100 ml. An injection of parathyroid extract (Parathormone) induced a phosphorus diuresis. The signs and symptoms of hypoparathyroidism rapidly became more pronounced. The patient had tingling sensation in the arms and legs, as well as carpopedal spasm. Nine days after the implantation of DCA pellets, he was found to have photophobia and episcleritis of both eyes. Therapy with vitamin D was begun

and continued for three days. Treatment was then changed to dihydrotachysterol (A.T.-10) in a dosage of 3 ml. daily, dosage being regulated by frequent Sulkowitch tests of the urine and serum chemical determinations. During a regimen of high calcium-low phosphorus diet, increasing doses of A.T.-10, oral calcium, and multiple intravenous doses of calcium gluconate, there was no appreciable change in serum calcium and phosphorus values, which remained in the range of 6.8 to 8.4 mg. per 100 ml. for calcium, and 7.2 to 5.0 mg. per 100 ml. for phosphorus.

There was no improvement in clinical symptoms. Muscular irritability and tetanic contractions continued. The patient became more antagonistic and belligerent and had several episodes of depression, during which he made two attempts to hang himself with bed clothing. He complained particularly of cramping pains in his legs, and of sharp cramping pain in the left costovertebral area. The possibilities of urinary-tract infection or calculus were considered but culture of the urine and intravenous pyelograms yielded negative findings. Following this period, as a result of efforts to combat his depressed emotional state by more personal attention and few disciplinary measures, he became more cheerful and cooperative, but showed no other objective evidence of improvement. Throughout the hospital stay the monilia infection of the mouth and nails was treated at various times with 10 per cent caprylic acid, Mycostatin, and 1 per cent gentian violet, with little improvement. Because of failure of A.T.-10 to affect the serum calcium and phosphorus levels, and because of the possibility that the patient had become resistant to this agent, A.T.-10 was discontinued and vitamin D (50,000 units per day) was given. In spite of the fact that this did not appreciably alter the serum calcium and phosphorus values (6.7 and 6.2 mg. per 100 ml., respectively), the patient's emotional status improved, and he requested that he be allowed to return to the orphanage. He was discharged on October 5, 1954, and told to take vitamin D (50,000 units per day), calcium lactate (10 grams per day) and cortisone (5 mg. three times per day). His discharge weight was 92 pounds.

He was seen in Endocrine Clinic on two occasions after discharge. A watery diarrhea of about 10 stools per day had developed, and he had become very careless about taking his medications. In spite of the diarrhea he gained about 2 pounds in weight and blood pressure readings were 120/80 and 120/90 mm. Hg. There were Chvostek signs on both occasions and Sulkowitch tests of the urine showed ++ to +++ values for calcium. He had no complaints except occasional muscle cramps and seemed to be feeling well.

At the orphanage, on October 24, 1954, he had generalized tetanic convulsions. These were treated with morphine by a physician who was not aware of the nature of his disease. The patient expired on October 25, 1954.

Autopsy

Autopsy was performed after the body had been embalmed. The skin was dark, particularly over the malar regions, elbows, wrists, knees and ankles. The hair of the scalp was very sparse; there was no beard and no axillary or pubic hair. The nails were rough, mottled and brown. There was pitting pretibial edema. Grossly, there were no lesions in the abdominal organs, except for fine nodularity of the surface of the liver; nor were there any lesions in the pleural and pericardial cavities, or the heart (weight, 195 grams). The thymus was enlarged, weighing 48 grams; on section, the tissue was gray-tan color and showed a normal lobular pattern. The thyroid appeared normal externally and on section; it weighed 8 grams. Despite detailed search of the neck tissues, no parathyroid glands were identified. The lungs were edematous and the trachea and major bronchi contained small amounts of aspirated gastric contents. The liver

weighed 1,200 grams and showed diffuse fine nodularity, both externally and on the cut surface, the lobules appearing to be surrounded by thin fibrous septa. There was no hemosiderosis or bile stasis, and there were no lesions in the gallbladder and bile ducts. The spleen was heavy (200 grams) and soft; there were many large malpighian follicles on the cut surface. There were unusually large lymphoid nodules in the stomach, small intestine and colon. The pancreas was not unusual externally or on section. The adrenals were small, gray and fibrous; each weighed 1 gram. There were no lesions in the kidneys, ureters and bladder, but the prostate, seminal vesicles and testes were unusually small. The brain was swollen (weight, 1,450 grams), having narrow sulci and flat gyri; sections showed distinct edema of the central cerebral white matter. The pituitary gland was not

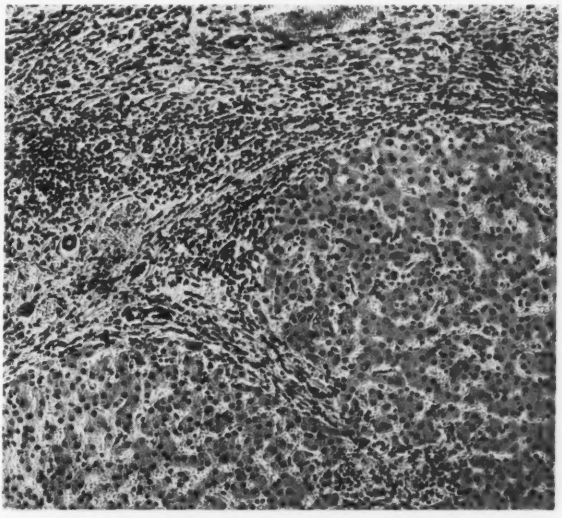

FIG. 3. Photomicrograph of liver, showing post-hepatitic scarring, with fine fibrous septa interrupting the lobular pattern, and lymphocytic infiltration of portal areas.

abnormal. The bones had very firm cortices, and soft red marrow. The lymph nodes of the cervical, mediastinal, hilar, mesenteric, retroperitoneal and pelvic groups were enlarged, discrete, firm and tan.

On *microscopic examination* the lungs showed early purulent bronchiolitis and bronchopneumonia, as well as foci of interstitial lymphocytic infiltration; there was no evidence of bronchopulmonary moniliasis. Foci of lymphocytic infiltration were also seen in the epicardium, gastric and small-intestinal mucosae, pancreas, hepatic portal areas, kidneys, adrenals, prostate, thyroid, cerebrum, cerebral arachnoid and anterior pituitary. The lymphoid nodules of the spleen, intestine and lymph nodes were hyperplastic and devoid of acute toxic degenerative changes. The cortex of the thymus also showed lymphocytic hyperplasia, microscopically resembling that of a much younger child. In the liver there was irregular fibrosis of portal areas, with slender fibrous septa separating clusters of lobules or surrounding single lobules (Fig. 3). No evidences of active hepatic cell degeneration, bile stasis, or bile-duct proliferation were seen. There was total cortical atrophy of the adrenals, with complete disappearance of the cortical epithelial

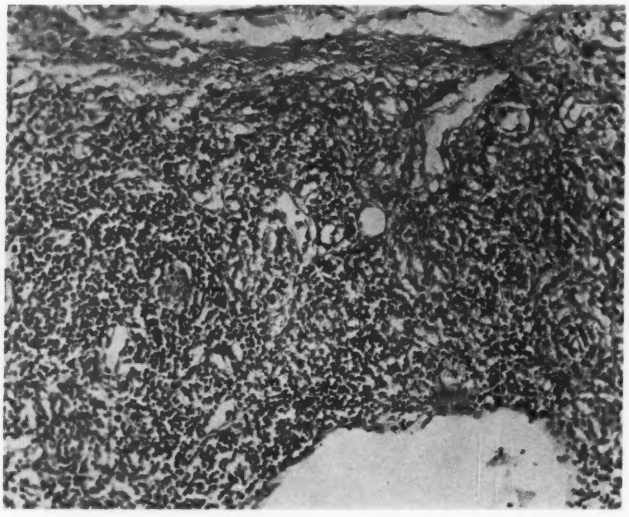

FIG. 4. Photomicrograph of adrenal, showing complete absence of cortical cells, and lymphocytic infiltration (heaviest in the medulla). The capsule is at the top and the central vein at the bottom on the photograph.

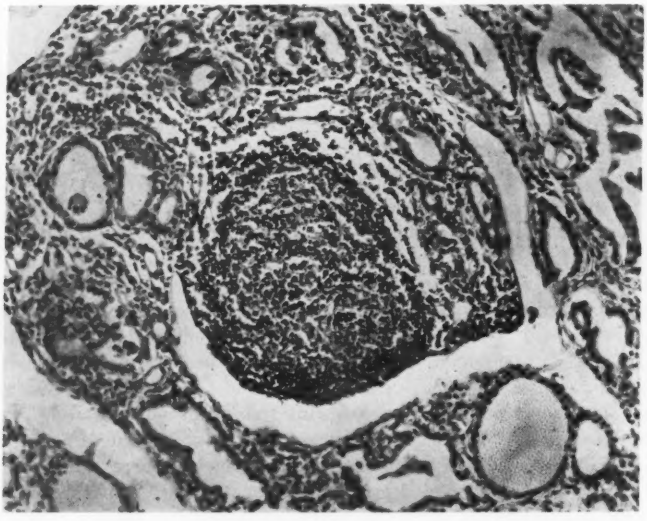

FIG. 5. Photomicrograph of thyroid, showing a lymphoid nodule, diffuse interstitial lymphocytic infiltration, lack of follicular colloid, and low follicular epithelium.

cells. The cortices were represented by thin layers of myxoid stroma containing variable numbers of lymphocytes, surrounded by thick connective tissue capsules (Fig. 4). The medullae were not abnormal in size, but were also infiltrated by lymphocytes, as were the retroperitoneal sympathetic ganglia. The lesion in the adrenals was considered to be the terminal state of "cytotoxic adrenal atrophy" (10, 11). In the thyroid, in addition to the foregoing type of focal lymphocytic infiltration, there were scattered lymphoid nodules with germinal centers, and diffuse colloid depletion; the epithelium of the follicles was low, and the appearance was that of secretory inactivity (Fig. 5). No parathyroid tissue was identified microscopically in several sections of neck organs. The prostate and testes were immature and prepubertal in character. In the bone marrow there was questionable hyperplasia of eosinophil cells and lymphocytes, but the bone spicules were microscopically normal. In the cerebrum and brain stem there were scattered lymphocytic infiltrates in the arachnoid membrane and the perivascular Virchow-Robin spaces, as well as in the basal ganglia. In the latter site there were also many small round laminated basophilic mineral concretions, chiefly situated in or near the sheaths of small blood vessels. The anterior pituitary showed, in addition to patchy lymphocytic infiltration, the typical changes of Addison's disease—pale degranulated basophil cells (12). There was no microscopic evidence of visceral moniliasis in any of the tissues.

The cause of death was considered to be acute hypoparathyroid tetany and cerebral edema, with acute bronchiolitis and bronchopneumonia due to aspiration of vomitus. Addisonian crisis and morphine intoxication may also have played roles; the marked sensitivity of patients with adrenal insufficiency to opium alkaloids is well known (13, 14).

DISCUSSION

The patient during life manifested the clinical features of superficial moniliasis, Addison's disease and hypoparathyroidism. Autopsy revealed apparent total atrophy of the parathyroid glands, total adrenocortical atrophy of the "cytotoxic" type, diffuse hepatic cirrhosis of the post-hepatitic type, and secretory inactivity of the thyroid similar to that seen in hypopituitarism. The case history and autopsy findings are presented as an example of a syndrome tentatively named "familial juvenile hypo-adrenocorticism, hypoparathyrodism and superficial moniliasis." Justification for the validity of this nomenclature follows.

From the available literature, information has been assembled on several cases of Addison's disease associated with hypoparathyroidism or superficial moniliasis, or both. In addition, data are presented on some siblings of these patients who showed only the association of two of these three conditions. The cases are tabulated briefly in Table 1. The apparent occurrence of this disease constellation among siblings in three families justifies the use of the term familial. The distribution does not imply that the disease pattern exhibited by these patients is genetically determined, since exposure of siblings to a common environmental factor may well have occurred. Among the patients listed in Table 1, there was none over the age of 10 years at the time of the earliest recorded manifestation of one

of the basic components of the syndrome. This fact justifies the term juvenile, even though 2 of the patients survived to the age of 20 years. Of the 13 patients listed in the Table 1, 8 had evidence of both Addison's disease and hypoparathyroidism, and 6 of these also had moniliasis. The occurrence of cirrhosis of the liver in the patient reported in this paper and in 1 of the patients reported by Craig, Schiff and Boone (2), suggests that

TABLE 1. SUMMARY OF DATA ON PATIENTS WITH HYPOPARATHYROIDISM, MONILIASIS, OR BOTH, ASSOCIATED WITH ADDISON'S DISEASE (INCLUDING DATA ON SIBLINGS WITH ANY OF THESE CONDITIONS)

Family No.	Reference No.	Case No.	Sex	Age at onset of			Outcome	Autopsy
				Moniliasis (years)	Hypoparathyroidism (years)	Addison's dis. (years)	(years)	
1	(7)	1	M	Infancy	10	—	Alive at 23	—
		2	F	3	6	—	Died at 14	No
		3	F	4–5	$11\frac{6}{12}$	? 12	Died at 12	No
2	(2)	1	M	$1\frac{6}{12}$	—	6	Died at 6	Yes
	(2, 8, 9)	2	F	1	7	$6\frac{6}{12}$	Died at 10	No
3	(2)	1	M	3	?	$11\frac{9}{12}$	Died at $11\frac{10}{12}$	Yes
	(2, 3)	2	M	$1\frac{6}{12}$	$6\frac{6}{12}$	—	Alive at 14	—
4	(2)	1	F	6	? $10\frac{2}{12}$	11	Died at $12\frac{2}{12}$	Yes
5	(1)	1	F	$2\frac{9}{12}$	$3\frac{2}{12}$	? $3\frac{8}{12}$	Died at $3\frac{8}{12}$	Yes
6	(4)	1	M	—	10	19	Alive at 20	—
7	(5)	1	F	—	$9\frac{6}{12}$	$10\frac{6}{12}$	Died at $11\frac{6}{12}$	Yes
8	(6)	1	M	? $5\frac{6}{12}$	9–10	10+	Alive at 10+	—
9	(this paper)	1	M	<2	$15\frac{6}{12}$	15	Died at $15\frac{10}{12}$	Yes

hepatic cirrhosis is another basic feature of this syndrome, but at present the data are not considered adequate to justify inclusion of cirrhosis in the name. Similarly, the possible significance of the sprue-like gastro-intestinal disorder observed in some of the patients listed in Table 1 cannot be assessed at present.

Of particular interest is the relation of the various components of the syndrome to each other. In all the patients in whom it was present, the superficial moniliasis preceded the hypoparathyroidism or Addison's disease (shortest interval, three months; longest interval, thirteen years). However the absence of evidence of visceral moniliasis in any of the 6

autopsy cases precludes the possibility that monilial infection of the parathyroids or adrenals led to the dysfunction of these glands. There is, further, no evidence that the various fungi of the genus Candida elaborate a toxin capable of producing such effects. If, in the 2 patients of Table 1 for whom skin or nail lesions were not described there was an actual absence of superficial moniliasis, this would also suggest that the moniliasis had no causal relationship to the subsequent endocrine disturbances. Although hyperadrenocorticism, either spontaneous or induced by administration of corticotropin or appropriate cortical steroids, appears to predispose patients to cutaneous or orificial moniliasis (? by the mechanism of "steroid diabetes"), there appears to be no evidence that hypoadrenocorticism has such an affect. This is supported by the lack of mention of moniliasis in a series of papers (15–23) on the familial occurrence of Addison's disease, studied in the hope of discovering other cases of the syndrome under discussion. The association of superficial moniliasis with hypoparathyroidism, whether sporadic or familial, is well known (2, 3, 7). The most probable explanation of the relationship between the moniliasis and the endocrine disturbances in the patients being considered is that the moniliasis was superimposed on dystrophic changes of the mucosae, skin and nails due to hypoparathyroidism; in these patients the dystrophic changes preceded by an unusually long interval the serum chemical changes denoting parathyroid insufficiency. This view is shared by Craig, Schiff and Boone (2) and is supported by the studies of Lachmann (24), but at present must be considered only an hypothesis.

The adrenal lesions in the patient described in this paper are typical of those seen in the form of adrenocortical atrophy called "cytotoxic" (10, 11). The cause of this condition is not known. The suggestion of Friedman (10), that the virus of infectious hepatitis may be the etiologic factor, is of interest in view of the occurrence of hepatic cirrhosis of the post-hepatitic type in our patient and of degenerative or cirrhotic liver lesions observed in several of the cases surveyed in Table 1; but, in the absence of routine methods for detection of antibody to this virus, no conclusion can be drawn at present. The presence of lymphocytic infiltrates in many organs in our patient is consistent with a diffuse viral infection in the past, but is more probably an aspect of the generalized lymphoid hyperplasia seen in Addison's disease, manifested in this patient also in the spleen, thymus, lymph nodes and lymphoid follicles of the intestine. Whether the sprue-like gastro-intestinal disorder observed in some of the patients listed is etiologically related to the hepatic lesions must also be considered, even though the reported liver changes do not suggest nutritional cirrhosis. The available data do not appear to indicate that liver damage is a basic feature of this syndrome, but this matter obviously requires further study. In the

absence of demonstrable parathyroid tissue, grossly and microscopically, no conclusions can be drawn from the present case as to the possible relationship between the parathyroid atrophy and the adrenocortical atrophy. The finding of parathyroid remnants in Cases 2 and 3 of Craig, Schiff and Boone's series (2) suggests, as does the relatively late age at onset of the serum chemical changes of hypoparathyroidism (range, $3\frac{2}{12}$ to $15\frac{6}{12}$ years; Table 1), that the parathyroid lesion is an acquired atrophy rather than a developmental hypoplasia or aplasia; but this is also an hypothesis. The lymphocytic infiltration with germinal center formation, secretory inactivity and colloid depletion of the thyroid in our patient suggest an early stage of Hashimoto's struma lymphomatosa, although the changes observed are not considered typical of that disease. Opinion is growing that struma lymphomatosa is the result of primary thyroid failure, rather than of chronic thyroiditis (25, 26). It thus seems possible that the syndrome under consideration should be considered one of multiple primary endocrine failure. More detailed study of affected patients, perhaps with increasing age, may produce more definite evidence of hypothyroidism, and thyroid failure may ultimately be added to the basic description of the syndrome. However, the interpretation that the thyroid changes are the result of lymphoid hyperplasia due to Addison's disease, and of pituitary dysfunction, is probably to be preferred at present. Whether or not clinically manifest thyroid failure occurs in this syndrome, the established association of failure of the adrenal cortices and parathyroid glands is puzzling, in that the anterior pituitary is generally considered as controlling the adrenals, but not the parathyroids. An analogous association is seen in the familial syndrome of adenomas of the pituitary, parathyroids and pancreatic islets (27, 28), the explanation of which is equally obscure. The immaturity of the testes in the patient described here may perhaps provide further evidence that pluriglandular primary endocrine failure is the basic feature of the syndrome under discussion. The testicular immaturity may, however, be only a nonspecific effect of retarded maturation consequent to adrenal and parathyroid insufficiency. Of interest in this connection, would be studies on gonadal function in patients with this disease-picture who attain greater age. The concept of primary endocrine failure can apparently not be extended to all endocrine glands, since none of the reported patients appears to have had lesions or dysfunction of the pancreatic islets.

Several authors of reports on the syndrome described in this paper have commented on amelioration of the symptoms of hypoparathyroidism with the onset of Addison's disease, or of exacerbation of the manifestations of parathyroid insufficiency as a result of hormonal treatment of adrenal insufficiency (2, 5, 6). Similarly, the administration of cortisone has been reported to interfere with the treatment of hypoparathyroidism in non-

addisonian patients, and to precipitate tetany in patients with calcium deprivation but normal parathyroid function (29, 30). More detailed discussion of the ways in which adrenal steroids may interfere with normal serum calcium-maintaining mechanisms is beyond the scope of this study. In our patient the clinical and chemical onset of hypoparathyroidism may well have been precipitated by the adequate hormonal therapy of Addison's disease. Thus patients with superficial moniliasis and Addison's disease must be watched closely when the hormonal therapy of the latter is begun, if severe manifestations of hypoparathyroidism are to be avoided. Similarly, children with hypoparathyroidism and moniliasis must be followed closely if acute adrenal insufficiency is to be prevented. In this regard, skin pigmentation and an increase in the color and number of pigmented nevi were very early signs of Addison's disease in several of the cases listed in Table 1. Both cerebral edema and focal mineralization of the basal ganglia, found at autopsy in our patient, are well known consequences of hypoparathyroidism (24, 31, 32). Neither mechanism is understood, but the terminal episode in this patient suggests that not only tetany but cerebral symptoms (perhaps analogous to those caused by the severe cerebral edema of lead poisoning), can develop in patients with hypoparathyroidism if treatment designed to maintain a normal serum calcium level is stopped abruptly. It also seems probable that the disturbed emotional state of the patient was in part due to these central nervous system manifestations of parathyroid insufficiency. Similar antisocial, depressive and paranoid traits have been described in hypoparathyroid patients; and the fact that this "toxic psychosis" may precede tetany or other manifestations of parathyroid insufficiency has been stressed by other workers (33, 34). In the presence of adrenal insufficiency, morphine is a particularly unfortunate choice of sedative. The mechanism by which patients with Addison's disease are rendered abnormally sensitive to the pharmacologic effects of the opium alkaloids is not clear, but prudence dictates that these drugs be administered only with great caution to children with hypoparathyroidism (who might well have hitherto undetected adrenal insufficiency).

SUMMARY

The clinical history, laboratory data and autopsy findings are presented in the case of a 15-year-old boy with Addison's disease due to cytotoxic adrenal atrophy, associated with hypoparathyroidism and superficial moniliasis.

Based on analysis of this case history, and the available data on 12 similar patients described in the literature, the suggestion is made that these patients represent a specific syndrome, tentatively called "familial

juvenile hypoadrenocorticism, hypoparathyroidism, and superficial moniliasis."

The possible relationships of the major components of the syndrome to each other, and to lesions in the liver, thyroid and gonads, is discussed. The available material permits no conclusion as to the etiology of the syndrome, except that visceral moniliasis is not the cause of the endocrine dysfunctions. The view is favored that the superficial moniliasis is the result of dystrophic lesions of the mucosae, skin and nails due to hypoparathyroidism, and that these lesions may precede chemical changes in the serum by a long interval.

The dangers of: (a) the development of Addison's disease in children with hypoparathyroidism and moniliasis, (b) the development of hypoparathyroidism in children with moniliasis and adrenal insufficiency, and (c) the administration of morphine to children with Addison's disease, are stressed.

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Letters to the Editor

EFFECTS OF THE SYNTHETIC ANABOLIC STEROID, 17-ETHYL-19-NORTESTOSTERONE, ON SERUM PROTEINS, LIPOPROTEINS AND LIPIDS IN HUMAN SUBJECTS*

To the Editor:

During an investigation of the metabolic effects of a new synthetic anabolic steroid, 17-ethyl-19-nortestosterone, in human subjects (1), definite alterations were observed in serum protein and lipoprotein fractions determined by paper electrophoresis. The sera of 10 patients with various clinical disorders were studied before, during and after daily intramuscular administration of 25 to 75 mg. of 17-ethyl-19-nortestosterone for five to thirty-five[1] days. In 8 of the 10 subjects, a marked increase in alpha-2 globulin and a significant but smaller increase in beta globulin (Fig. 1) occurred. One subject exhibited an increase only in alpha-2 globulin, and 1 (whose control serum alpha-2 globulin level was abnormally elevated) exhibited no change in serum protein patterns when the steroid was administered. Quantitative colorimetric determination of each protein fraction was carried out on 0.2 N NaOH eluates of the sectioned paper electrophoretic strips of 5 subjects, by a modification of the method of Kunkel and Tiselius (2) (Table 1). Alpha-2 globulin, estimated by this procedure, increased from 24 to 46 per cent and beta globulin increased from 14 to 40 per cent in these sera.

In 7 of the 10 subjects, steroid administration was associated with a definite increase in beta lipoprotein, demonstrated by staining the strips for lipid with oil red 0. In strips from 2 of the 7, a disappearance of the alpha-2 lipoprotein band was also observed. In 1, the beta-lipoprotein changes were equivocal and in 1, absent. Serum lipoprotein fractions decreased in the subject who showed no protein change.

Total lipid, lipid phosphorus, and total cholesterol concentrations were determined by standard methods (3) in 8 of the 10 subjects. In 4,

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[1] One subject who initially was given 50 mg. a day for three weeks received 25 mg. every other day for three months.

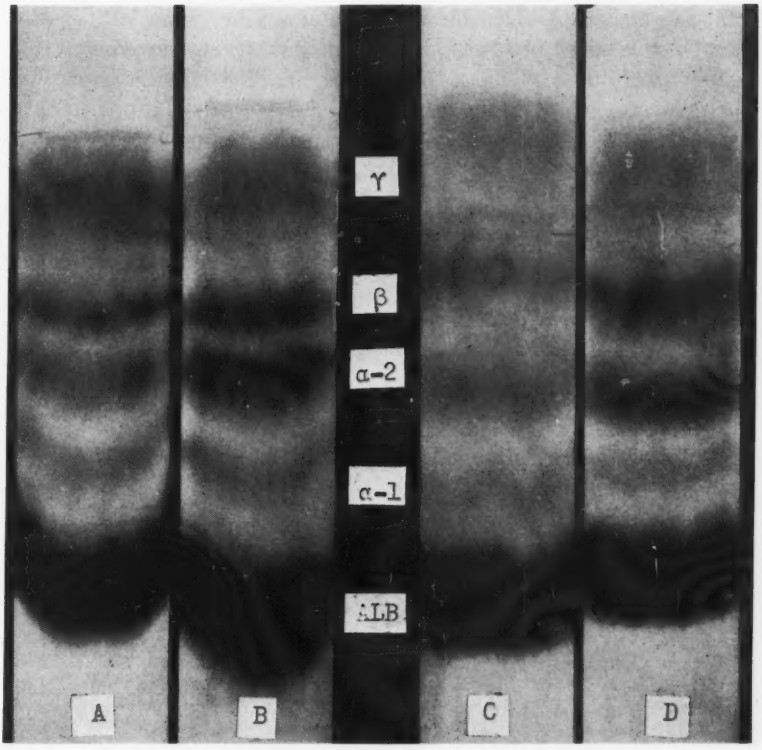

FIG. 1. Effect of administration of 17-ethyl-19-nortestosterone on serum proteins as determined by paper electrophoresis. **A** and **C** represent serum patterns before administration of the steroid to 2 subjects; **B** and **D**, after administration of the steroid. Note the marked increases in alpha-2 and beta globulin.

there was a definite increase in all of the chemically determined fractions, and in 2 there was no change. The 2 patients whose serum lipid levels decreased were the subjects who exhibited either no change or a decrease in the concentration of serum lipoproteins. Both of these patients died of their primary disease within two and one half weeks after the study was completed.

These effects upon serum proteins and lipoproteins may be observed as early as three days after beginning administration of the steroid and apparently parallel the other anabolic effects. When maximum anabolic effects occurred after the steroid was discontinued, the greatest changes in protein and lipoprotein were also noted at the same time.

The well-documented lower incidence of coronary artery disease in young women, in contrast to that in men or in postmenopausal women, has led

to great interest in the possible influence of sex hormones on serum lipid and lipoprotein partition. Russ, Eder and Barr (4) separated serum alpha and beta-lipoproteins by Cohn fractionation and found that young women have a greater concentration of total cholesterol in the alpha-lipoprotein fraction than do young men. Moreover, when patients who have survived

TABLE 1. EFFECT OF 17-ETHYL-19-NORTESTOSTERONE ON SERUM PROTEIN FRACTIONS

Patient	Period	Albumin (Gm. %)	Globulin (Gm. %)			
			Alpha-1	Alpha-2	Beta	Gamma
1						
	Control	3.40	.423	.565	.626	1.41
	Steroid	3.23	.344	.607	.731	1.34
	Steroid	3.34	.299	.755	.725	1.34
	Post-steroid	3.58	.348	.689	.874	1.44
2						
	Control	3.59	.429	.645	.829	1.17
	Steroid	4.10	.356	.845	.965	1.00
	Post-steroid	3.84	.354	.570	.937	1.15
	Post-steroid	3.69	.403	.606	.967	.96
3						
	Control	3.41	.333	.657	.700	1.35
	Steroid	3.03	.384	.942	.799	1.38
	Post-steroid	3.65	.446	.735	.740	1.11
4						
	Control	3.22	.301	.894	.961	.905
	Steroid	3.58	.178	1.040	.658	.865
	Post-steroid	3.37	.287	1.100	.777	1.000
	Post-steroid	3.07	.334	1.110	1.220	.925
5						
	Control	3.14	.372	.87	1.09	1.60
	Steroid	3.02	.340	1.22	1.24	1.14
	Post-steroid	3.09	.382	1.27	1.13	1.36

myocardial infarctions are given estrogens, the abnormally increased cholesterol concentrations in their beta-lipoproteins and their elevated serum total cholesterol concentrations decrease toward normal. When estrogen therapy is discontinued, the abnormal serum lipid pattern promptly reappears. Conversely, testosterone induces an increase in beta-lipoprotein and less consistent changes in the level of chemically-determined lipids.

The synthetic steroid, 17-ethyl-19-nortestosterone, has been found to be highly anabolic but much less androgenic than testosterone in human

subjects (1). In fact, the typical menstrual periods exhibited by 2 postmenopausal women (1) ten days after stopping the injections would suggest an estrogenic effect which may reflect some *in vivo* metabolic transformation of this steroid. The action of 17-ethyl-19-nortestosterone on serum lipoproteins resembles that of testosterone. However, to our knowledge, there have been no reports of a marked increase in alpha-2 globulin concentration following administration of any steroid. The purpose of this report is to call the attention of other investigators to this phenomenon. Further studies on the changes in serum lipoprotein partition in man, as determined by Cohn fractionation and ultracentrifugal analysis, are planned.

CONCLUSION

The synthetic anabolic steroid, 17-ethyl-19-nortestosterone, induced significant increases in serum alpha-2 and beta-globulins in 9 of 10 human subjects. In 8 subjects there were accompanying increases in beta-lipoproteins, and in 4 of these, increases in the levels of chemically determined serum lipids were also noted. It is suggested that further studies of this phenomenon are indicated, particularly of the hitherto unreported increase in alpha-2 globulin following administration of steroid.

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TRANSFER OF THYROID HORMONE ACROSS THE HUMAN PLACENTA AT TERM*

To the Editor:

Surprisingly little information is available concerning the placental transfer of thyroid hormone in human subjects (1). Two previous studies report conflicting results and are inconclusive (2, 3). More definitive data (recently reviewed by Peterson and Young (4)) have been obtained from animal experiments, which suggest species differences in the permeability of the placenta to this hormone (4–7). Placental passage has been reported in the rat, guinea pig and rabbit (4–6) but, in an unconfirmed study, transfer was not demonstrated in the dog (7).

The present study was designed to evaluate more precisely the ability of thyroid hormone to cross the human placenta.

Methods. A dose of 10–15 microcuries of I^{131}-labelled *l*-thyroxine or *l*-triiodothyronine[1] was administered intravenously to 15 pregnant subjects, at intervals varying from ten minutes to one hundred and sixty-nine hours before spontaneous vaginal delivery or elective cesarean section. Prior to the administration of labelled thyroid hormone, sodium iodide was injected intravenously to prevent thyroidal uptake of the sodium iodide^{131} liberated by metabolism of the radioactive hormones. The iodide salt was continued by mouth when delivery was delayed beyond six hours. This technique effectively prevented the concentration of I^{131} in the fetal thyroid, since the measurements of radioactivity over the anterior neck and the thigh of these infants did not differ significantly. Maternal venous blood was collected ten minutes after the injection of labelled hormone and also at the moment of delivery, and blood was obtained from the umbilical vein immediately after clamping the cord. The specimens were examined for serum total radioactivity and serum precipitable (organically-bound) radioactivity (SPI^{131})—the latter after trichloracetic-acid precipitation by the standard procedure (8). Radioactivity measurements were performed in a heavily shielded well-type scintillation counter. Samples of amniotic fluid were also assayed by this method.

Results. Values obtained following injection of labelled thyroxine are listed in Table 1. It may be seen that there was a progressive fall in the maternal/fetal ratio of SPI^{131}. This is attributable in part to the gradual transfer of organically bound I^{131} across the placental barrier.

A limited number of studies were made using labelled triiodothyronine (Table 2). With this compound, the fetal serum organic radioactivity

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[1] The I^{131} labelled *l*-thyroxine and *l*-triiodothyronine were obtained from Abbott Laboratories, Oak Ridge, Tennessee.

TABLE 1. PLACENTAL TRANSFER OF *l*-THYROXINE-I^{131}

Subject	No. of hours before delivery that T_4I^{131}* was injected i.v.	Maternal/fetal ratio SPI^{131} at delivery	$\frac{SPI^{131} \text{ at delivery}}{SPI^{131} \text{ 10 min. after injection}} \times 100$†	
			Maternal	Fetal
C.K.‡	2.0	12.6	83.2	6.6
M.F.§	2.2	44.1	75.3	1.7
R.F.	2.8	39.5	95.1	2.4
G.P.	7.2	18.9	77.6	4.1
M.J.	8.3	22.0	69.6	3.2
P.C.	8.5	15.7	79.3	5.1
V.W.§	18.8	7.7	54.2	7.1
M.C.§	70.5	3.1	43.5	13.8
M.B.	169.0	2.1	28.2	13.4

* *l*-Radiothyroxine.
† Corrected for physical decay.
‡ Active labor at time of injection.
§ Cesarean section.

AMNIOTIC FLUID:

Ratio SPI^{131} am. fluid/cord serum	
V.W. (18.8 hrs.)	0.03
M.C. (70.5 hrs.)	0.16

constituted a significantly smaller percentage of the serum total radioactivity than following the administration of labelled thyroxine.

Discussion. The presence of SPI^{131} in the mother and the fetus cannot be attributed to the secretion of thyroid hormones labelled *in vivo*, since the uptake of I^{131} by the maternal and fetal thyroids was effectively prevented by the large amounts of inorganic iodide administered to the mother. It is assumed that the trichloracetic-acid precipitable I^{131} in the fetal serum was predominantly contained in the administered labelled *l*-thyroxine or *l*-

TABLE 2. PLACENTAL TRANSFER OF *l*-TRIIODOTHYRONINE-I^{131}

Subject	No. of hours before delivery that T_3I^{131}* was injected i.v.	Maternal/fetal ratio SPI^{131} at delivery	$\frac{SPI^{131} \text{ at delivery}}{SPI^{131} \text{ 10 min. after injection}} \times 100$	
			Maternal	Fetal
A.R.†	0.16	224.2	—	—
C. de S.	7.5	9.9	31.7	3.2
R.O.	8.8	9.7	24.6	2.5
E.G.	10.7	5.5	52.3	9.5
G.V.	10.8	4.7	19.0	4.0
V.Wi.‡	16.3	7.2	15.8	2.2

* *l*-Radiotriiodothyronine.
† Active labor at time of injection.
‡ Cesarean section.

triiodothyronine. In view of the slow rate of passage, it is possible, however, that a significant fraction of the fetal SPI^{131} was composed of metabolites of thyroxine or triiodothyronine, such as recently described by Roche *et al.* (10). Chromatographic identification has not yet been accomplished.

The results indicate that, although the human placenta is relatively impermeable to thyroxine at term, a significant quantity of this hormone is transported across it. The rate of transfer from mother to fetus is slow, and in these experiments appeared to level off between 18.8 hours and 169.0 hours before delivery. However, the data are insufficient to establish that complete equilibration had been achieved between the circulating labelled thyroxine in the maternal blood and that in the fetal blood. Binding of *l*-thyroxine in the maternal circulation by plasma proteins, particularly thyroxine-binding protein in the α-globulin fraction (9), may explain the delay observed in placental transfer.

In the subjects injected with I^{131}-labelled triiodothyronine, the maternal/fetal ratio of SPI^{131} and the maternal level of SPI^{131} at delivery decreased more rapidly than with labelled thyroxine. If the rate of metabolism of triiodothyronine is comparable in the mother and the fetus, the placental transport of triiodothyronine appears to be more rapid than that of thyroxine. This difference may be a property of the less intense binding of triiodothyronine to plasma proteins (9).

These observations have a bearing upon certain clinical features of cretinism. In general, completely athyreotic sporadic cretins do not present obvious signs of thyroid insufficiency during the first few weeks of life. Nevertheless, stigmata of fetal deprivation of thyroid hormone may be present: 1) a retarded level of osseous development, which is below that for full-term newborn infants, sometimes associated with epiphyseal dysgenesis in the prenatal ossification centers, and 2) persistent mental retardation and neurologic defects despite early institution of thyroid therapy—presumably due to injury of the central nervous system incurred prenatally.

Conclusion. From the results of this study, it is concluded that the human placenta is capable of transmitting significant amounts of thyroid hormone but the rate of transfer across this "barrier" is slow. Therefore, the quantity of hormone transferred from the mother may be insufficient to support normal skeletal and brain development when the fetal contribution is deficient because of a primary thyroidal defect.

More complete details will be reported elsewhere.

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THE ACTIVITY OF *dl*-TRIBROMTHYRONINE

To the Editor:

Since it was found that *l*-triiodothyronine is the most active of the thyroid hormones, it became of interest to speculate on the activity of other tri-halogen derivatives of thyronine. Figure 1 depicts the results of assay of *dl*-tribromthyronine in 2 patients with myxedema. The results indicate that 1.2 mg. of *dl*-tribromthyronine is equivalent to 0.75 mg. of *dl*-thyroxine. Thus, thyroxine is one and one-half times as strong as tribromthyronine on the basis of weight, or two and one-half times as strong on the basis of equivalent weights. These results agree with those reported by Compston and Pitt-Rivers (1).

Since previous reports have indicated the relative activities of thyroxine versus tetrabromthyronine (2) and of thyroxine versus triiodothyronine

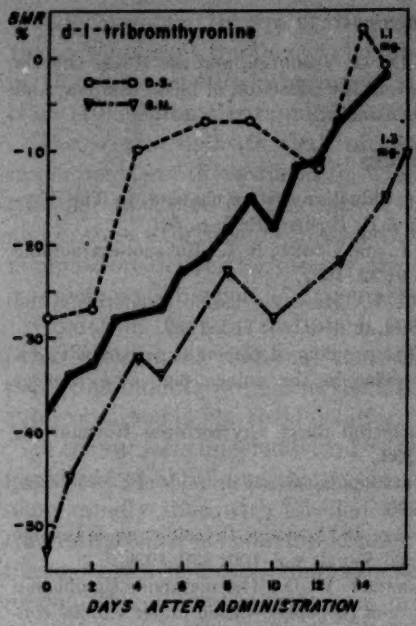

FIG. 1. Response of 2 patients with myxedema to the daily intravenous administration of *dl*-tribromthyronine. The first patient, D. S., received an average daily dose of 1.1 mg.; the second patient, G. M., received 1.0 mg. daily for ten days, 1.5 mg. daily for three days and 2.0 mg. daily for three days, the average daily dose being 1.3 mg. The heavy line is the standard response of patients with myxedema to the daily administration of 0.5 mg. of iodine equivalent to 0.75 mg. of *dl*-thyroxine.

TABLE 1. RELATIVE ACTIVITIES OF SOME IODINE AND BROMINE DERIVATIVES OF THYRONINE, ON THE BASIS OF EQUIVALENT WEIGHTS

Thyroxine	17	times	Tetrabromthyronine
Thyroxine	2.5	times	Tribromthyronine
Tribromthyronine	7	times	Tetrabromthyronine
Triiodothyronine	4	times	Thyroxine
Triiodothyronine	10	times	Tribromthyronine

(3), it is of interest to compare the relative activities of all these compounds as shown in Table 1.

The relatively high activity of tribromthyronine suggests that it may be useful under certain conditions, clinical or experimental, when a highly active compound is desirable and when the presence of iodine is to be avoided.

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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Eleanor Venning, Royal Victoria Hospital, Montreal, Quebec, Canada, *not later than February 1, 1957. It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that these will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program and journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. Reference, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*,

who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.

4. Abstracts should be letter perfect, since there will be no opportunity for proof reading by authors.

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should

be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE EIGHTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY; THE UNIVERSITY OF TEXAS POSTGRADUATE SCHOOL OF MEDICINE; AND THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, HOUSTON, TEXAS

October 22–27, 1956

The faculty will consist of twenty-two eminent clinicians and investigators from various parts of the country in the field of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program and registration, write to *Office of the Dean, The University of Texas, Postgraduate School of Medicine, Texas Medical Center, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

(For Faculty and Program *see* the August issue of this Journal.)

U. S. PUBLIC HEALTH SERVICE GRANTS FOR TRAINING IN STEROID INVESTIGATION

Recognizing the increasing importance of steroids in many fields of medical research, the United States Public Health Service has awarded grants making possible a program to provide specialized training for individuals interested in steroid investigation. The National Cancer Institute of the National Institutes of Health has established grants for a joint training program in steroid biochemistry to be conducted at Worcester, Massachusetts and at Salt Lake City, Utah through the cooperative effort of the Worcester Foundation for Experimental Biology with the Department of Chemistry, Clark University and the Department of Biochemistry, College of Medicine, University of Utah. Two types of fellowships are planned: a one-year program carrying a stipend of $5000, for individuals having a B.S. or M.S. degree in chemistry or in medical technology, or an equivalent, which would carry a stipend of $1500 for the six-month period. The post-doctoral fellowship is intended for scientists who contemplate research involving steroid techniques and who wish to obtain a broad experience. During a portion of the year such fellows may initiate work on their particular problems under the guidance of established investigators. The pre-doctoral program is primarily intended to train individuals in the analytic determination of various steroids. Trainees completing the six-month program will be able to carry out such determinations competently, either in connection with a research program or for diagnostic purposes. They should also be in a position to master new techniques as they appear. Both training programs will include theoretical and practical considerations as well as experience in specialized techniques. The first groups will begin *January 1, 1957*, at Salt Lake City, closing date for application *November 1*; and *February 1, 1957* at Worcester, Mass., closing date for application *November 1, 1956*. Individuals or institutions wishing to obtain more information regarding this program should communicate directly with Dr. Kris Eik-Nes, Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City or Dr. Frank Ungar, Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957.*

THE VAN METER PRIZE AWARD FOR 1957

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in the Hotel Statler, New York, May 28, 29 and 30, 1957, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, *not later than January 15, 1957.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend. The essay will be published in the annual proceedings of the Association.

BOOK REGISTER

Psychiatry, The Press and The Public. Problems in Communication. Edited by Winfred Bloomberg, M.D., Chairman; L. Lyons, R. T. Morris, R. L. Robinson, A. Snider and S. B. Hanau. Summary of a Conference held in Greenfield, Massachusetts, June 24–26, 1955, under the auspices of the American Psychiatric Association in cooperation with the National Association of Science Writers and the Nieman Foundation for Journalism; 80 pages; 1955. Washington, D.C., American Psychiatric Association; Price $1.50.

Radioactive Iodine in the Diagnosis and Treatment of Thyroid Diseases (in English). By Dr. Carlos Blanco-Soler, in collaboration with Drs. José Otto, Carlos Blanco Soler y Ros, Alberto Salgado, and Isaac Mandelbaum Asma, Madrid, Spain; 103 pages; 12 figures; 1955. Madrid-Lisboa.

Research in Psychosomatic Medicine. Psychiatric Research Report No. 3—American Psychiatric Association. Consultant editor, Nathan S. Kline, M.D., Director of Research, Rockland State Hospital, Orangeburg, N. Y. Papers presented at the Southern Regional Research Conference held under the joint auspices of the American Psychiatric Association and Duke University School of Medicine, Durham, N. C., November 19–20, 1954, including symposia on Problems in the Laboratory Study of Emotions and Bodily Function; Problems in the Measurement, Testing, and Rating of Psychological and Social Functioning; and Problems in Establishing a Psychodynamic Explanation; 99 pages; 1955. American Psychiatric Association, 1785 Massachusetts Avenue, N.W., Washington, D. C.

Therapeutic Use of Artificial Radioisotopes. Edited by Paul Harris, Ph.D., Professor of Experimental Oncology and Director of Cancer Research Laboratories, Meharry Medical College, Nashville, Tenn. Written by internationally recognized specialists, this is an up-to-date guide to the treatment of virtually all benign and malignant conditions which are susceptible to therapy with radioactive isotopes; 424 pages; illustrated; 1956. John Wiley & Sons, Inc., 440 Fourth Avenue, New York, N. Y. Price $10.00.

Therapy of Fungus Diseases. Edited by Thomas H. Sternberg, M.D., Professor of Medicine, and Victor D. Newcomer, M.D., Associate Professor of Medicine. An International Symposium presented June 23, 24 and 25, 1955 by The Division of Dermatology, Department of Medicine, School of Medicine and Medical Extension, University of California at Los Angeles; constituting a review of the world's knowledge of fungus infections, particularly with regard to therapy. Biologic aspects are also discussed; 360 pages; illustrated; 1955. Little, Brown and Company, Boston, Mass. Price $7.50.

Transactions of the American Goiter Association for 1955. A collection of 35 papers and discussions dealing with clinical and biochemical aspects of the thyroid problem. Clinicians and research workers may well regard this yearly volume as a source book for information concerning the latest advances in therapy and physiology of the thyroid, in articles written by foremost authorities in the field; 482 pages; illustrated; 1956. Charles C Thomas, Publisher, Springfield, Illinois. Price $14.00.